HILGARDIA

A Journal of Agricultural Science Published by the California Agricultural Experiment Station

VOL. 13 JANUARY, 1941 No. 10

EXPERIMENTS WITH FUNGICIDES FOR USE AGAINST SCLEROTIUM ROLFSII IN SOILS[1, 2]

A. E. DAVEY[3] AND L. D. LEACH[4]

INTRODUCTION

IN THE EARLY PHASES of the investigation of measures for control of southern sclerotium rot of sugar beets, caused by *Sclerotium Rolfsii* Sacc., among other lines of attack much attention was given the possibility of destroying the fungus in the soil. It was thought at that time that the fungus might be eradicated by timely application of fungicides. Now that the wide distribution of the disease and the extent of infested tracts in the Sacramento Valley have been determined, workers have realized that such an attack must have limited value.

The sclerotia of this organism are, however, discrete bodies, uniform and convenient in size, furnishing admirable material for the study of the effectiveness of fungicides. It is thought that the following work may show the relative value of several such chemicals and may aid investigators working with less easily handled organisms.

THE EFFECT OF HYDROGEN-ION CONCENTRATION ON THE DEVELOPMENT OF SCLEROTIUM ROLFSII

The Influence of Hydrogen-Ion Concentration of the Medium on the Growth of Sclerotium Rolfsii on Agar Plates.—The more conspicuous characteristics of the behavior of this fungus in culture have been reported by Higgins (*5*),[5] who found that measurable growth takes place

[1] Received for publication October 10, 1939.

[2] These investigations were made possible largely through financial contributions by the beet-sugar companies operating in California.

[3] Instructor in Pomology and Junior Pomologist in the Experiment Station.

[4] Associate Professor of Plant Pathology and Associate Plant Pathologist in the Experiment Station.

[5] Italic numbers in parentheses refer to "Literature Cited" at the end of this paper.

in standard beef-extract peptone broth with 2 per cent saccharose between the limits of pH 1.4 and pH 8.8. In media (standard broth) having a limited carbohydrate supply but differing in pH, the fungus uniformly produced an acidity of pH 4.0 except in media originally of extreme acidity—that is, greater than pH 2.0. On the other hand, where as much as 2 per cent saccharose was added to the broth the end point was changed to approximately pH 5.0, except for media originally more acid than pH 2.0. This, Higgins found to result from the ability of the fungus to produce large quantities of oxalic acid.

On solid media containing bromcresol purple we have found that acid produced by the mycelium diffuses into the agar in advance of the growing hyphae. Within the limits of pH 8.0 and pH 2.0 the fungus can grow freely, perhaps overcoming deleterious effects from the original reaction of the medium by its acid-producing ability; or, on carbohydrate-poor media of low pH, it can produce basic material, possibly ammonia as suggested by Higgins (*5*).

In our experiments, however, at pH 8.0 a solution of 82.4 millimols of K_2SO_4 per liter of water saturated with calcium carbonate had not killed mycelium of *Sclerotium Rolfsii* immersed in it for five days. Since no growth and no change in reaction occurred, evidently a sustained reaction of pH 8.0 in itself cannot be very toxic to even the vegetative form of the fungus. The absence of growth of the mycelium in this solution would result from lack of nutrients even if there were no inhibitory effect of the solution.

The Influence of Hydrogen-Ion Concentration in Soils on the Occurrence of Southern Sclerotium Rot in Beet Fields.—Considering the laboratory evidence just cited one can hardly expect that the hydrogen-ion concentration in soils producing profitable crops of sugar beets will itself markedly affect the development of the fungus and aid in reducing the losses in beet crops. That such is the case, has been verified by field observations. In several fields, the percentage of diseased plants was estimated in certain areas, and the pH of soil samples taken within these areas was determined. Losses of 50 per cent or more were observed in three different fields where the pH of the soil was between 7.6 and 7.7, and in one field a loss as great as 20 per cent occurred in an area where the soil reaction reached pH 8.2. Although the disease was usually more severe in areas of low pH, there was no conclusive evidence that the alkalinity of the soil—up to pH 8.0—limited the distribution of *Sclerotium Rolfsii* or strikingly reduced the severity of the disease.

The Effect of Liming Soil on Infection of Carrots.—In the past, several workers have limed the soil in the hope of reducing losses caused by root parasites. The results, however, have been very variable. Higgins

(*6*) showed that some control of the bed rot of sweet potatoes caused by *Sclerotium Rolfsii* was obtained by liming except where excessive organic matter was present. Wellman (*15*) found club root of crucifers to occur in the field irrespective of the pH of the soil. Although he markedly reduced the amount of disease in a cabbage field by using 2 tons of calcium hydroxide, he believed this to be the result of the specific toxic action of the calcium hydroxide toward *Plasmodiophora brassicae* Wor., inasmuch as calcium carbonate and calcium sulfate were not satisfactory inhibitors.

TABLE 1

EFFECT OF CALCIUM HYDROXIDE ON SOIL REACTION AND ON INFECTION OF CARROTS IN THREE SOILS

Soil	Lime added		pH of soil	Number of carrots tested	Number of carrots infected
	Per cent of weight of soil	Pounds per acre			
Sacramento clay from Reclamation District 999	None	None	6.9	12	9
	0.037	1,480	7.7	6	5
	0.074	2,960	7.8	6	6
	0.185	7,400	7.9	6	3
	0.370	14,800	8.1	6	6
Sacramento clay from Reclamation District 1660	None	None	7.3	12	7
	0.370	14,800	7.8	17	6
	0.740	29,600	8.5	17	4
	1.480	59,200	8.8	17	0
Sacramento clay from Reclamation District 999	..	None	7.5	21	21
	..	10,000*	8.0	18	18

* Application of lime in the field and artificial infestation with *Sclerotium Rolfsii*.

His results somewhat resembled the experience herein reported regarding the southern sclerotium rot in nontreated soils of various pH values and regarding the apparent benefit secured by liming.

Besides field observations on the relation between the pH of unaltered soils and infection of sugar beets, calcium hydroxide was used for shifting the reaction of similar soils to determine whether this change would inhibit somewhat the infection of carrot roots in these soils under greenhouse conditions. Soil from two infested fields was air-dried, and portions were mixed with amounts of calcium hydroxide. Thus a series was obtained in which the content of $Ca(OH)_2$ ranged from none to 0.371 per cent of the air-dried weight of the soil, in the first case, and from none to 1.480 per cent in the second. The maximum amounts in these two trials were equivalent, respectively, to applications of 14,800 and 59,200 pounds of calcium hydroxide per acre. In addition, soil from two uninfested fields, treated with 10,000 pounds of lime per acre one and two

years previously, was seeded with sclerotia. Samples of these soils were placed in pots in the greenhouse and, after they had absorbed water from beneath for five days, were planted with carrot roots. As table 1 reveals, though the alkalinity of the soil was increased, there is no evidence that infection was inhibited by liming, except where the equivalent of about 30 tons of lime per acre was applied and a pH of 8.8 was produced.

Effect of Liming Soil on Infection of Sugar Beets.—Calcium hydroxide was applied in field experiments that included tests of several other materials. The application (5 tons per acre) was made to $\frac{1}{40}$-acre plots in randomized blocks, with five replications. The stand of beets and percentage of infected beets were irregular in these plots so that the reduc-

TABLE 2

The Effect Upon Losses in the Sugar-Beet Crop of Applying Calcium Hydroxide to Infested Soil

	Range of pH of soil	Average pH in soil	Infected beets, per cent	Yield of disease-free beets, tons per acre
Lime-treated	7.0–8.1	7.46	45.48	10.92
Control	6.7–7.3	7.10	59.32	7.81
Difference	.....	0.36	13.84	3.11
Difference for significance, 19:1 odds	.....	....	14.60	2.85
Difference for significance, 99:1 odds	.....	....	20.12	3.92
Calculated F value	.....	....	7.03	11.95

tion in the average percentage of infected beets in treated plots is a little less than the difference required for significance with 19:1 odds as determined by the analysis of variance (*13*); but the increase in average yield, although significant when 19:1 odds are accepted, is not significant with 99:1 odds (table 2).

Most of the fields in northern California where *Sclerotium Rolfsii* is abundant are neutral or slightly alkaline in reaction. Under such conditions, small or moderate applications of lime produce no striking shift in reaction.

THE TOXICITY OF VARIOUS FUNGICIDES IN AQUEOUS SOLUTION

Among fungicides, formaldehyde has been popular because of its general adaptability in plant-disease control, especially where soil organisms have been involved. It may be used, therefore, as a standard for reference in placing a value on other fungicides. In the present work it has been thus treated to some extent, but with no attempt to calculate a "formaldehyde coefficient" in the sense of the "phenol coefficient." The

results given in table 3, having been obtained under uniform conditions, are comparable, although they have been collected from work extending over several years. Sclerotia from pure cultures on potato-dextrose agar were immersed in lots of 50 enclosed in cheesecloth. At the end of the test period each lot was washed in three changes of distilled water, plated on potato-dextrose agar, and incubated at 30° C for five days. The tests were conducted at room temperature.

As will be noted, the test periods are longer than those usually re-

TABLE 3

THE TOXICITY TO SCLEROTIA OF VARIOUS FUNGICIDES IN WATER SOLUTION

Materials	Fungicide, parts per million of solution	Minimum lethal exposure, in hours
Acetic acid	10,000	1
Ammonia (NH_3)	350	12
Ammonia (NH_3)	700	2
Ammonium thiocyanate	2,000	Not killed in 24 hours
Boric acid	10,000	Not killed in 2 hours
Calcium cyanamid (commercial)	2,400*	120
Chlorinated lime in water	546†	10
CS_2 emulsion	3,250‡	2
Ca $(OH)_2$	1,000§	6
Dowicide B	500	6
Dowicide H	500	1
Dowicide P	500	6
Formaldehyde (HCHO)	955	12
Formaldehyde (HCHO)	3,700	2
Mercuric chloride	1,000	¼
Phenol	500	2
Potassium ethyl xanthate	475‡	Not killed in 23 hours
Pyroligneous acid	10,000	Not killed in 2 hours
Sodium hydroxide	400	12

* Estimated available $CaCN_2$; solution of pH 10 determined by glass electrode.
† Available chlorine—titrated with sodium thiosulfate.
‡ Estimated available CS_2 in commercial preparation.
§ Saturated solution of pH 12 determined by glass electrode.

ported in such work. This condition is inevitable because of the size (diameter 0.5–2.0 millimeters) and the compact structure of the sclerotial bodies. With most materials in concentrations which might be used in practice, several hours are required for penetration to the point of killing all the tissue of the sclerotia. Since mercuric chloride is apparently an exception to this rule, the writers believe that it may, if absorbed in superficial tissue, prevent the emergence of mycelium from deeper living cells. The data presented are for the shortest period that resulted in the killing of all sclerotia in the test lot.

Of all the materials tested, only ammonia was definitely superior to formaldehyde in point of toxic power and cost, though carbon disulfide

emulsion proved fully as toxic as formaldehyde. The relative toxicity of ammonia and formaldehyde to *Sclerotium Rolfsii* has been previously reported (*7*) in some detail; and Oserkowsky (*11*) gives information on the toxicity of several other fungicides to *S. Rolfsii*.

Tests on Infested Soil in Greenhouse Flats.—Early in these investigations—September to November, 1932—tests were made of applications of formalin, ammonium hydroxide, carbon disulfide emulsion, mercuric chloride, and acetic acid solutions to naturally infested Sacramento clay loam. This soil had a moisture equivalent of 51 per cent. For the experiments, 2,800 grams of air-dry soil, in one series of tests, and 2,400 in the other, were made up to half the moisture-holding capacity and placed in small 8-inch flats to a depth of 2–3 inches. The solutions of chemicals were added to the surface of the soil in amounts (1,680 and 1,500 cubic centimeters) slightly exceeding the moisture-holding capacity—that is, at the rate of 1.14 and 1.02 gallons, respectively, per square foot of soil surface. Excess water was drained off through a false screen bottom, and the soil allowed to stand for 10 days. To determine the relative efficiency of the fungicides, samples of sclerotia recovered by washing the soil through a 40-mesh screen were tested for germinability, as described elsewhere (*8*). After being placed in cheesecloth the sclerotia were surface-sterilized in bichloride of mercury, 1:1,000, for 45 seconds, washed in sterile water, and plated on potato-dextrose agar. In one series of tests, sclerotia from the surface of the soil in the flats were plated separately.

The results (table 4) definitely indicate the relatively high efficiency of formaldehyde and the failure of the other treatments to kill sclerotia even at depths of soil as shallow as 2 or 3 inches.

In one case where a 1:400 dilution of formalin (37 per cent formaldehyde) was used, the sclerotia had so deteriorated that only 16 were recovered from one flat, whereas the soil from the other flats yielded well over 100 sclerotia each after treatment.

Where the sclerotia were recovered from the surface of the soil, none of those treated with formalin (1:100 and 1:400) or with bichloride of mercury (1:1,000) were found viable. Those surface sclerotia that were recovered after treatment with carbon disulfide emulsion (1:100 and 1:400) and with Diesel oil proved on plating to be 94.6, 97.8, and 59.3 per cent viable, respectively.

Depths to Which Formaldehyde, Ammonia, and Sodium Hydroxide Solutions Are Effective When Applied to the Surface of the Soil.—Field applications of solutions of formaldehyde, ammonia, and sodium hydroxide were made in the summer of 1933 (July–September) in three representative districts in the Sacramento Valley. In the test with Yolo loam at Davis, samples of sclerotia were placed at 6-inch and 12-inch depths

before treatment, whereas the other two experiments were on naturally infested soils from which diseased beets had just been removed. The rates of application were 1½ and 3 gallons per square foot on plots 4 square feet in area. The formalin solutions used ranged in strength from 1 volume of commercial formalin (37 per cent formaldehyde) in 50 volumes of water to 1 volume in 1,000. Ammonia water (28 per cent anhydrous ammonia) was diluted to 100 volumes with water. Sodium

TABLE 4

THE EFFECTIVENESS OF AQUEOUS SOLUTIONS OF SEVERAL FUNGICIDES AS WELL AS CARBON DISULFIDE EMULSION AND DIESEL OIL IN KILLING SCLEROTIA IN SOIL

Material	Dilution ratio	Application of the pure chemicals, pounds per acre	Number of trials	Number of sclerotia plated	Viable sclerotia, per cent
Formalin (37 per cent HCHO)	1:100	1,530	3	335	1.2
	1:200	765	2	252	2.0
	1:400	382	3	276	2.8
	1:1,000	153	2	238	22.7
Ammonium hydroxide (28 per cent NH_3)	1:60	960	2	100	10.0
	1:180	320	1	50	74.0
	1:360	160	1	50	76.0
Acetic acid (glacial)	1:100	1,875	1	50	28.0
Mercuric chloride (dry salt)	1:1,000	167	1	50	48.0
CS_2 emulsion (65 per cent CS_2)	1:100	1,088	1	50	82.0
	1:400	272	1	50	80.0
Diesel oil (sp. gr. 0.87)	Without dilution	416,000	1	50	66.0
Control H_2O	..	..	3	297	88.9

hydroxide solution was made up by dissolving 1 part by weight in 100 parts of water.

The soil was ridged up around the plots to provide a basin. Careful excavating after an application of 3 gallons of 1:100 formalin revealed a limited lateral spread (6 inches) of the solution beyond the measured boundary of the plot, and a corresponding reduction of the depth of penetration in the marginal zone of the treated areas to 16 inches, as compared with 21 inches in the middle of the plot. The plots in the sugar-beet fields were sampled with a soil tube 8 to 10 days after treatment in horizons of 0–6 inches and 6–15 inches. As previous trials (*8*) had shown, a very small proportion of the sclerotia in an infested field are to be found below 15 inches, most of them being located in the surface 6 to 8 inches. These soil samples were washed through a 40-mesh screen in the laboratory,

TABLE 5

THE EFFECTIVENESS OF FIELD APPLICATIONS OF AQUEOUS SOLUTIONS OF FUNGICIDES IN KILLING SCLEROTIA

Chemical	Soil type and locality	Gallons applied per sq. ft.	Sampling horizon, inches	Per cent* of sclerotia viable after treatment of soil with solutions of the given strengths					
				1:50	1:100	1:200	1:400	1:1,000	Controls H_2O
Formalin (38 per cent HCHO)	Yolo loam, Davis	3	0–6	..	0	74	72	94	32
			6–15	..	..	..	..	..	62†
	Sacramento clay, Clarksburg	3	0–6	..	0	32	64	91	..
			6–15	..	6	8	56	94	..
	Sacramento clay, Clarksburg	1½	0–6	..	0	24	38	96	..
			6–15	..	34	46	80	90	98†
	Sacramento clay, Woodland	3	0–6	6	6	2	14	80	..
			6–15	64	37	50	71	93	87†
Aqua ammonia (28 per cent NH_3)	Yolo loam, Davis	3	0–6	..	76	..	..	..	32
			6–15	..	..	..	..	..	62
	Sacramento clay, Woodland	3	0–6	..	94	..	..	..	..
			6–15	..	92	..	..	..	87†
Sodium hydroxide (1 per cent NaOH)	Sacramento clay, Woodland	3	0–6	..	70	..	..	..	..
			6–15	..	78	..	..	..	87†

* Percentages based on samples of 50 sclerotia recovered from the soil 10 days after treatment. (In eight cases less than 50 sclerotia were recovered.)
† Horizon 0 – 12 inches rather than 6 – 15 inches.

and samples of 40 to 50 sclerotia were plated on potato-dextrose agar after surface sterilization for 45 seconds with 1:1,000 $HgCl_2$. In 8 tests out of the 59, fewer than 50 sclerotia were recovered; and in these cases all the sclerotia were plated.

Sclerotia placed in soil before treatment were made up in lots of 50 and tied in cheesecloth before being planted at depths of 6 inches and 12 inches. The soil was thoroughly packed around the samples after placement.

Table 5 gives the percentage of viable sclerotia in the various test samples. As will be noticed, a 1–100 dilution of commercial formalin was highly effective to a depth of 6 inches, but not lower. More dilute solutions had little effect on the viability of sclerotia. Aqua ammonia and sodium hydroxide diluted 1–100 were not lethal to sclerotia in soil.

To complete the information given in table 5, we must mention the development upon plated sclerotia of other organisms apparently growing from within the mercuric-chloride-treated surface. These organisms were characteristically of two sorts: first, a white bacterium appearing consistently upon sclerotia recovered from control plots wet only with water; second, a *Trichoderma* species appearing upon considerable numbers of the sclerotia treated with formalin. The presence of the bacteria or fungi may have considerably affected the percentage germination given above, for the controls in the experiments with Yolo loam soil showed a lower percentage of viable sclerotia than did the treatments with 1:1,000 dilution of formalin. In this instance, out of the 50 nontreated sclerotia recovered from the 6-inch level, 34 did not germinate; and from all these 34 there grew the white bacterium. That a higher percentage of germinated sclerotia might have been secured is suggested by the more recent experience of Leach and Mead (*9*), who indicate a preference for plating on peat soil without sterilization.

The relation of *Trichoderma* to sclerotia from soil treated with formalin is even more striking. The percentage of sclerotia from which *Trichoderma* grew decreased along with the strength of the solution used for treating the soil. In other words, *Trichoderma* apparently involved the interiors of the sclerotia as they became weakened or killed, the invader finally becoming deeply seated enough to be below the zone affected by the surface application of mercuric chloride. The data, showing the effect of applying 1½ gallons per square foot, are given in table 6.

Judging from these results *Trichoderma,* instead of being an active parasite of *Sclerotium Rolfsii,* invades only the sclerotia killed or weakened by chemical treatment. This opinion is supported by the fact that *Trichoderma* is only occasionally recovered from either viable or nonviable sclerotia washed from nontreated soils.

Experiments with Formalin in Preventing Plant-to-Plant Spread of the Rot in Field Rows of Sugar Beets.—Frequently, in fields where southern sclerotium rot occurs, infected plants are found adjacent in the rows, suggesting that the disease has been transmitted by growth of the causal fungus through the soil. If such be the case, removal of single affected plants as soon as possible after symptoms appear might be expected to effect a material saving in the crop, especially if the operation were accompanied by treatment of the soil from which the plants were removed. The logic of the procedure seems well founded, since the extensive development of mycelium in the soil depends upon the presence of abundant nutritive material such as is furnished by the sugar beet.

TABLE 6

The Relation of Strength of Formalin Solution to Germination of Sclerotia and to Invasion of Sclerotia by Trichoderma

Strengths of formalin solutions used in treating soil	Per cent sclerotia recovered from soil	
	Germinating	Showing growth of *Trichoderma* on plating
1:100	8.5	57.0
1:200	30.8	21.4
1:400	64.8	15.8
1:1,000	86.5	3.0
Control	98.0	0.0

The method was tested in June, 1934, by a field experiment in the Holland land tract. Because the crop was subirrigated, there was no carrying of sclerotia such as might occur with surface water moving along the rows. In this field, at the start of the experiment, approximately 100 plants per acre or 0.4 per cent of the stand exhibited symptoms of the disease.

The experimental area was divided by the irrigation ditches into three plots of about the same size (1.5 acres). In one plot the diseased plants were located and marked with stakes but not removed, and no formalin was applied to the soil. In a second plot some 150 diseased plants were located and removed, together with the soil immediately surrounding each diseased beet root. After removal of each root, 3 quarts of a solution consisting of 1 part of commercial formalin to 50 of water were poured into the space previously occupied by the beet. In a third plot 20 infected plants were found and removed, and the adjacent soil treated with 2 gallons of 1:50 formalin, the disinfectant being retained by 12-inch

TABLE 7

THE EFFECT OF REMOVING DISEASED BEETS FROM THE ROW, WITH SUBSEQUENT LOCAL SOIL STERILIZATION, UPON THE INFECTION OF ADJACENT BEETS

Procedure	Number of diseased plants	Plants becoming infected within 20 days after removal or marking of adjacent diseased plants	
		In same row	In next row
Plot 1: Plants not removed; soil untreated	57	23	12
Plot 2: Plants removed; soil treated with 3 quarts formalin (1:50) per plant	83	17	13
Plot 3: Plants removed; soil treated with 2 gallons formalin (1:50) per plant	20	3	13

metal cylinders open at both ends, the lower edge being thrust into the soil. On June 25, twenty days after treatment, counts were made of the numbers of diseased plants adjacent in the same row, and in the rows next on each side, to spots from which plants had been removed, as compared with the numbers of diseased plants found adjacent to those pre-

TABLE 8

THE EFFECTIVENESS OF FORMALIN IN KILLING SCLEROTIA IN INFESTED SOIL* FROM WHICH DISEASED PLANTS HAVE BEEN REMOVED

Treatment and plant number		Sclerotia recovered per 200 grams of soil	Per cent germination
Plants not removed; untreated	1	333	100
	2	165	98
	3	915	98
	4	572	100
	5	585	98
	Average	*514*	*98*
Plants removed; space treated with 3 quarts formalin (1:50)	1	63	0
	2	21	0
	3	43	2
	4	20	0
	5	55	48
	Average	*40.4*	*10*
Plants removed; space cylinder-treated with 2 gallons formalin per diseased plant	1	249	0
	2	2	0
	3	46	0
	4	67	0
	5	252	0
	Average	*123.2*	*0*

* Soil sampled to a depth of about 1 foot.

viously marked as diseased, but not removed. The numbers of diseased plants subsequently occurring in the same row as the removed or marked plants show (table 7) that the amount of disease was reduced as the result of treatment or removal. The spread to plants in adjacent rows appeared, however, not to be affected by the methods used.

In the field where these trials were conducted the percentage of diseased plants was, as will be noted, very small. Where the disease is more abundant the methods used in this experiment could be of little practical value.

At the time of the final counts, soil samples were taken from several treated spots and also from untreated areas around diseased plants that had not been removed. Sclerotia recovered by washing the soil samples through a screen were plated on potato-dextrose agar after surface sterilization for 45 seconds with 1:1,000 $HgCl_2$. Table 8 shows the effectiveness of the treatments in reducing the number of viable sclerotia left in the treated areas. Evidently, where the disease is localized in a very small area, such timely treatment of diseased plants may reduce the amount of infective material left in the soil. Probably, where as much as 90 per cent of the sclerotia in the treated spots are killed, as in the present case, the incidence of the disease in subsequent crops would be considerably reduced.

THE TOXICITY OF VOLATILE FUNGICIDES TO SCLEROTIA

The Toxicity of Ammonia, Formaldehyde, Naphthalene, Xylene, Tetrachlorethane, Pentachlorethane, Chloropicrin, and Carbon Disulfide to Wet and Dry Sclerotia.—Since Oserkowsky (*11*) found chloropicrin, tetrachlorethane, and pentachlorethane ineffective against *dry* sclerotia and since Godfrey (*4*) has pointed out that *wet* sclerotia are readily killed by chloropicrin, all these materials were tested against air-dry sclerotia and against sclerotia soaked in water for at least 15 minutes before treatment.

Sclerotia were subjected to the vapors of ammonia and formaldehyde over aqueous solutions; the solutions of ammonia contained 0.14, 0.07, and 0.028 per cent of NH_3, and the formaldehyde solutions 0.4, 0.2, 0.1, and 0.04 per cent of formaldehyde. Tests of naphthalene, xylene, tetrachlorethane, pentachlorethane, chloropicrin, and carbon disulfide were conducted with saturated atmospheres over the surface of the chemical.

The sclerotia used in testing the ammonia and formaldehyde solutions were from plate cultures on potato-dextrose agar. For the other materials the sclerotia were obtained from field-infected sugar beets. Those produced on beets are characteristically larger than those from plate cultures.

After treatment with gaseous ammonia and formaldehyde, the sclerotia were plated directly on potato-dextrose agar. In the other tests they were plated on unsterilized peat soil as described by Leach and Mead (*9*).

Tests were run for 24 and 72 hours at room temperature in 22-mm test tubes, fitted with rubber stoppers except that with chloropicrin and carbon disulfide, glue-coated corks were used. Sclerotia were in cheesecloth sacks suspended above the chemicals.

The results of the experiment, presented in table 9, show that sclerotia

TABLE 9

THE TOXICITY OF VAPORS OF VOLATILE FUNGICIDES TO WET AND DRY SCLEROTIA

Material	24 hours' exposure				72 hours' exposure			
	Wet sclerotia		Dry sclerotia		Wet sclerotia		Dry sclerotia	
	Plated	Viable	Plated	Viable	Plated	Viable	Plated	Viable
Ammonia (NH_3)								
0.14 per cent solution	20	0	20	0	..	..	..	..
0.07 per cent solution	20	2	20	13	20	0	20	2
0.028 per cent solution	20	20	20	20	20	20	20	19
Formaldehyde (HCHO)								
0.4 per cent solution	20	0	20	0	..	..	..	..
0.2 per cent solution	20	5	20	1	20	0	20	0
0.1 per cent solution	20	19	20	18	20	0	20	0
0.04 per cent solution	20	20	20	19	20	19	20	20
Naphthalene	40	24	40	22	..	..	..	..
Xylene	19	16	19	18	..	..	..	..
Tetrachlorethane	19	0	19	19	..	..	..	..
Pentachlorethane	21	0	20	19	..	..	..	..
Chloropicrin	50	0	50	50	..	..	..	..
Carbon disulfide	50	0	50	3	..	..	..	..

were killed by vapors of the more concentrated solutions of both ammonia and formaldehyde. There is some indication that wet sclerotia were more readily killed by ammonia vapors than dry sclerotia. Evidently, however, moisture-saturated atmospheres at room temperature will supply sufficient moisture to make ammonia an effective killing agent. Further information on this point is furnished by another experiment in which air-dry sclerotia were placed in suction flasks, which were evacuated to give a pressure of 38 centimeters of mercury (about ½ atmosphere). Anhydrous ammonia was then run into the flask until atmospheric pressure was restored. In the first of two trials all the sclerotia were killed after three hours' exposure, but in the second, 10 sclerotia out of a sample of 50 remained viable.

Again table 9 clearly shows the difference in the susceptibility of the

wet and dry sclerotia to chloropicrin, tetrachlorethane, and pentachlorethane.

A striking feature of the data is found in the fact that sclerotia (both wet and dry) were extremely resistant to vapors of xylene and naphthalene; indeed those of the latter have been found according to data not included in table 9 as merely inhibiting growth without further apparent injury to the fungus even after 5 days' exposure. This was surprising because of the effectiveness of xylene reported by Ezekiel and Taubenhaus (*2*) in field tests with *Phymatotrichum omnivorum* and because of the results of Weiss and Evinger (*14*), who found that naphthalene vapor kills sclerotia in 3 to 4 days' exposure at 30° C. In a later experiment, however, we have found that air-dry sclerotia soaked for only 15

TABLE 10

Effectiveness of Xylene Against Sclerotia Soaked in Water for Different Periods Previous to Treatment

Time sclerotia soaked in water	Per cent germinated after:		
	24 hours' over xylene	48 hours' over xylene	1 week over xylene
Dry sclerotia	100	100	76
15 minutes	38	14	0
45 minutes	28	4	0
3 hours	0	0	0

minutes in water are still relatively resistant to exposures of 48 hours to saturated vapors of xylene, as are those soaked for as long as 45 minutes; whereas sclerotia soaked for 3 hours were all killed by an exposure as short as 24 hours (table 10).

Just why the dry sclerotia must be soaked so long before the beginning of the test is a point of interest. We assumed that initiation of growth processes might be necessary. The recent experience of Pinckard and his associates (*12*), however, concerning the increased toxicity of benzene vapors to tobacco leaves after wetting opens the question of the extent to which water had penetrated the sclerotial "tissue" within the shorter periods of soaking. Thus if the toxicity of the fungicide were to depend upon its accumulating in the water in contact with the cells, one might judge that only the outer sclerotial cells were wet, leaving the interior dry and not exposed to the fungicide in the condition in which it could be effective. These points we have not had the opportunity to investigate.

The Effectiveness of Anhydrous Ammonia Against Sclerotia in Soils of Different Moisture Content.—With a view to injecting anhydrous

ammonia as a volatile fungicide into infested soils, we investigated, as follows, the physical condition of the soil necessary to the effective use of that chemical. Seemingly, if the gas could be made to permeate dry soil and kill sclerotia, there would be reasonable hope for its practical application.

To establish the lower limit of soil moisture necessary to the effective use of anhydrous ammonia against sclerotia, two soil types were investigated: a Yolo fine sandy loam with moisture equivalent of about 17 per cent, and Columbia silty clay loam with a moisture equivalent of 28 per cent. Test samples of sclerotia were placed in a median position in columns of 30 grams of oven-dry soil. The columns were made up by placing

TABLE 11

THE RELATION OF THE MOISTURE CONTENT OF SOIL TO THE EFFECTIVENESS OF ANHYDROUS AMMONIA IN KILLING SCLEROTIA

Moisture content of soil	Per cent of sclerotia surviving treatment						
	After 2 hours' exposure in:		After 6 hours' exposure in:		After 12 hours' exposure in:		After 24 hours' exposure in Columbia silty clay loam
	Yolo fine sandy loam	Columbia silty clay loam	Yolo fine sandy loam	Columbia silty clay loam	Yolo fine sandy loam	Columbia silty clay loam	
Oven dry	..	64	..	48	36	18	14
5 per cent	100	96	0	0	0	0	0
12.5 per cent	91	98	0	0	0	0	0
27.3 per cent	..	94	..	100	..	96	60

the soil in a short length of 22-mm glass tubing, the ends of which were closed with cheesecloth to retain the soil. The moisture contents of other samples of the same soil were made up to 5 per cent, 12.5 per cent, and 27.3 per cent, the last being close to the field capacity of the Columbia silty clay loam. The tubes were placed in 2-liter suction flasks (one to a flask); and, as in the previous experiment, ammonia to give a partial pressure of ¼ atmosphere was introduced by replacement. The results on plating the sclerotia after different periods of treatment are given in table 11, which shows the percentage survival in samples of 50 sclerotia. Evidently, air-dry sclerotia in contact with soil containing 5 per cent soil moisture are rendered susceptible to the relatively heavy dosage employed. The crumb structure of the soil containing 27.3 per cent moisture was broken down in the process of packing the tube, and the plug thus made prevented killing by the gas within the time of the exposure.

Conceivably, since such a relatively low moisture content of the soil would render the use of ammonia effective, one might utilize anhydrous

ammonia in the field by injecting it into the soil. Accordingly a galvanized iron cylinder with a perforated bottom, a diameter of 12 inches, and a depth of 24 inches was obtained. A large sample of Columbia silty clay loam, dried to a moisture content of 7 per cent, was screened through 0.25-inch mesh. A piece of glass tubing was bent into a 10-inch circle and pierced at eight points, spaced equally along its length, by holes about 1 mm in diameter. One end was sealed, the other attached to a rubber tubing that led from a cylinder of anhydrous ammonia. The flow of ammonia from the cylinder was controlled by a needle valve calibrated (by collecting the gas over saturated Diesel oil) to deliver an average of 364 cubic centimeters in 20 seconds. In setting up the experiment, four test lots consisting of 35 to 60 sclerotia in cheesecloth were distributed in each

TABLE 12

Killing Range of Anhydrous Ammonia in Air-Dry Soil

Distance from level at which NH_3 was introduced, centimeters	Per cent of viable sclerotia found 20 hours after introducing NH_3							
	Above level of introduction				Below level of introduction			
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 1	Sample 2	Sample 3	Sample 4
Same level	0	0	0	0	..	..	..	..
7.6	0	0	0	0	0	0	0	0
15.2	83	76	82	77	10	0	86	0
22.8	63	85	82	77	32	34	35	39

of seven horizontal planes separated by 3.45 kilograms of soil at vertical distances of about three inches. The delivery tube was set in the median horizontal plane. When the experiment was thus set up, the needle valve was opened to deliver ammonia for 91 minutes at the rate indicated. Thus there was introduced some 80 liters of gas or an estimated 60 grams of ammonia, at standard conditions of temperature and pressure. There was no convenient way of checking the actual delivery; but because of the constant pressure in the tank it is supposed not to differ greatly from expectation.

Twenty hours after starting the delivery of ammonia, the test samples of sclerotia were recovered from the soil, surface-sterilized for 45 seconds in 1:1,000 $HgCl_2$, washed, and plated on potato-dextrose agar. Table 12 shows the percentage of viable sclerotia at the various distances from the level at which the ammonia was introduced.

Thus, as will be seen, a volume of ammonia (80 liters) several times the approximate pore space (24 liters) of the volume of soil (37 liters) used was insufficient to penetrate the mass of soil even with as little as 7 per cent moisture. The rate of injection was slow, somewhat less than a

liter per minute, because of the need for controlling the amount used. It is unknown what might be the result of using the much greater rates of delivery possible, and thus perhaps forcing the gas through the soil mass more rapidly than the soil could absorb it.

Laboratory Tests of the Toxicity of Vapors of Xylene, Tetrachlorethane, Pentachlorethane, Chloropicrin, and Carbon Disulfide to Sclerotia in Soil.—The literature regarding the use of volatile disinfectants in soils has been reviewed by Bliss (*1*).

A simple technique was adopted for these tests: a weighed amount of soil, adjusted to the desired moisture content by the method of Nichols (*10*), was placed in quart Mason jars; and the material to be tested was introduced from a graduated pipette through a glass tube which was placed vertically in the jar at the time of filling the latter with soil and which reached to the bottom of the jar.

Immediately after introduction of the fungicide, the tubes were sealed at the upper end with cork stoppers freshly coated with animal glue. The jars were sealed with glass tops and rubber rings after vapor clinging about the top of the jar had been blown away and after sclerotia soaked for at least 15 minutes had been placed on small squares of cheesecloth on top of the soil. This procedure resembles that used by Ezekiel and Taubenhaus (*2*) in tests with *Phymatotrichum.*

The soil-moisture content was adjusted to three levels: 10 to 11 per cent, 15 to 17 per cent, and 23 to 25 per cent. The last approached the moisture equivalent of the sample of Columbia silty clay loam used. The jar was completely filled by 970 grams of oven-dry soil made up to between 23 and 24 per cent soil moisture. With less soil moisture, the jars were not quite filled. In some tests with chloropicrin, samples of screenings from two beet-receiving stations were used. These were passed through a 4-mesh screen to remove large parts of sugar beets and leafy material. Although these samples possibly included several soil types and certainly contained undetermined amounts of organic matter, the results did not differ markedly from those obtained with the Columbia silty clay loam. Most of the tests were made at room temperature; others in a thermostat at 25° C. The time between introduction of the fungicide and recovery of sclerotia from the jars was 24 or 48 hours. Sclerotia used for these tests were obtained from diseased sugar beets and were produced either in the laboratory or in the field. After exposure to the test materials, the sclerotia were plated on peat soil without surface sterilization and were incubated at 30° C. The tests were repeated at least twice, usually three and four times, with close agreement. The figures presented in table 13 are for a single representative trial with soil at 10 per cent and 25 per cent moisture, and a test period of 48 hours.

Chloropicrin is conspicuously more effective than any of the other materials. Carbon disulfide, which approaches it most closely, is only about one fifth as toxic. Xylene, tetrachlorethane, and pentachlorethane were definitely ineffective under the experimental conditions. Here we must recall that the sclerotia were soaked for 15 minutes before being placed in the jars. As previously stated, it has since been learned that the duration of soaking the sclerotia within the range used in the experiment is of vital importance to the results. Possibly, therefore, these three fumigants would have been much more effective if the sclerotia had been

TABLE 13

The Comparative Effectiveness of Five Volatile Fungicides Against Sclerotia Exposed for 48 Hours in Soil in Closed Glass Containers

Fungicides	Per cent moisture in soil	P.p.m. of fungicide in dry soil	Sclerotia in test sample	Viable sclerotia
Xylene	11	10,000	20	20
	25	10,000	20	19
Tetrachlorethane	11	5,000	20	20
	25	5,000	15	15
Pentachlorethane	11	5,000	20	20
	25	5,000	20	15
Chloropicrin	11	100	20	0
	11	50	20	20
	25	100	40	0
	25	50	40	40
Carbon disulfide	10	450	40	19
	23	500	40	12
	23	600	40	0

soaked for at least 3 hours. Nevertheless the dosage of fumigant used sufficed to saturate the space within the jar in each case; and sclerotia similarly treated except for the presence of soil succumbed to both tetrachlorethane and pentachlorethane, though not to xylene. The soil has therefore undoubtedly reduced their effectiveness by restricting diffusion, and probably by absorption. Variations in moisture content of the soil did not change the results. It should also be pointed out that the soil moisture was sufficient in all cases to saturate the atmosphere within the jars with water vapor.

Depth to Which Sclerotia Are Killed by Chloropicrin in Soil When the Gas Is Confined at the Surface.—Though the tests in Mason jars offer a convenient means of comparing different fungicides and soils, the values obtained for such a small, confined space can scarcely serve as a basis for dosage in the field, where continuous diffusion and downward movement

by convection take place in the soil. The following experiment, accordingly, was set up in the laboratory with chloropicrin as the fungicide.

A cylinder was made of a special chloropicrin-proof kraft paper supplied by Dr. G. H. Godfrey, who has investigated the efficiency of such materials (*3*). This paper consists of two thicknesses glued together and has one side treated with paraffin. In making up the cylinder a strip 24 inches wide was wrapped around a section of 3-inch pipe, and the free edge secured by Scotch masking tape. The top and bottom of the tube were made of the same paper, the bottom being folded over the paper at the end of the pipe and secured by more tape.

Upon removal of the paper cylinder from the pipe, the paper tube was filled with fractional amounts of Columbia silty clay loam made up to a moisture content of 15 per cent. As each fraction of 1,165 grams of soil was placed in the tubes and tamped firmly, a test sample of 40 sclerotia was placed on the leveled soil surface. Thus the sclerotia were spaced at intervals very close to 15 centimeters. When the tube had been filled with four such fractions, the last sample of sclerotia was covered with a centimeter depth of soil; and the top end of the tube was closed by a cap secured by masking tape.

The dosage of chloropicrin was calculated on the basis of 2 cubic centimeters per square foot of soil surface. This requires 0.136 cubic centimeters of fungicide for the 63.6 square centimeters of cross-sectional area of the tube. The chemical was applied by means of a 2-cc glass hypodermic syringe with a 2.6-cm needle. A puncture was made in the tube 16 centimeters from the upper end; and after the injection of the chemical on the insertion of the full length of the needle, this puncture was closed with a small piece of Scotch tape. In actually making the injection, a slight excess or 0.14 cubic centimeter of chloropicrin was introduced. The delivery of the hypodermic according to the graduation of the cylinder was found to have an extreme error of $\pm$ 3 per cent when checked by weighing, on a chemical balance, the amount delivered. The tube was stood upright after introduction of the chloropicrin.

The sclerotia were recovered from the tube after 48 hours by sectioning the tube and soil column with a large knife at the points where the test samples had been placed, starting with the lowermost samples. As each cut was made, the soil surface exposed was carefully examined for chloropicrin odor. The sclerotia recovered were immediately plated on peat. Table 14 gives results of the examination and plating.

The chloropicrin is of course very easily detectable in extremely low concentration by smell. Evidently little of the vapor, if any, penetrated as far as 30 centimeters. On the other hand, sclerotia were killed at distances of 15 centimeters above and below the points of injection. An ap-

plication of 2½ cc per square foot of soil surface injected to a depth of 6 inches should then be adequate to kill sclerotia to a depth of 12 inches with proper confinement of the gas at the surface. Apparently, furthermore, downward movement of the vapors by displacement of air is very slight with such small doses and within the limited depths of soil under treatment.

The Effectiveness of Chloropicrin in Killing Sclerotia When Applied to an Infested Area in a Beet Field.—In August, 1934, 400 square feet of sugar-beet field on Grand Island, Sacramento County, was treated with chloropicrin. Nearly all the beets in this area had been killed by *Sclerotium Rolfsii*, and the rotted roots were removed before treatment. The soil, belonging to the Egbert series, appeared dry and friable. The

TABLE 14

The Killing Range of Chloropicrin in Soil Confined 48 Hours in a Glued Paper Cylinder

Distance of sclerotia from point of injection, centimeters	Per cent of sclerotia found viable	Chloropicrin detectable
15 above	0	Strong odor
0	0	Strong odor
15 below	0	Strong odor
30 below	67	No odor
45 below	98	No odor

surface soil was worked into loose condition by hand-digging, and half the area was moistened by applying a quart of water per square foot before and again after working of the soil. By means of a Vermorel applicator 1-cc amounts of chloropicrin were injected at intervals of 1 foot each way to a depth of 6 inches over the entire area. The total amount delivered was checked by measuring the amount of residue in the applicator. As the area was treated, the surface was covered with kraft wrapping paper sized on both sides with 10 per cent animal glue as suggested by Godfrey (*3*). As successive strips of paper were laid down over the treated areas. their edges were secured by Scotch masking tape. The edges of the paper at the periphery of the area were carefully buried under several inches of soil.

Two days after the application of the chloropicrin the cover was found to have been badly whipped by the wind. There was at least one considerable tear, and the Scotch tape had pulled loose in several places. The confinement of the gas was therefore not complete.

Samples of sclerotia were recovered from the treated area to a depth of 8 inches by screening them out of a number of soil-tube cores taken

both before and after the chloropicrin was applied. The sclerotia were surface-sterilized with 1:1,000 $HgCl_2$ for 45 seconds before being plated on potato-dextrose agar. The results of viability tests of these sclerotia appear in table 15. Evidently, the percentage of viable sclerotia was reduced from 90 to between 20 and 30. The sclerotia from the wetted area showed a slightly lower percentage of germination than those from the dry areas.

The dosage of 1 cubic centimeter per square foot of soil, if assumed to penetrate uniformly to a depth of 1 foot, would be at the rate of about 60 parts per million of dry soil. This is less than the effective rate (100 parts per million of dry soil) with nearly perfect confinement of the gas under laboratory conditions and is only half the dose found necessary

TABLE 15

THE VIABILITY OF SCLEROTIA FROM A FIELD PLOT TREATED WITH CHLOROPICRIN AT THE RATE OF ONE CUBIC CENTIMETER PER SQUARE FOOT OF SOIL SURFACE

Samples	Number of sclerotia plated	Number of sclerotia viable	Per cent of sclerotia viable
Sclerotia from soil samples taken before treatment	200	181	90.5
Sclerotia from soil samples taken two days after treatment, soil left dry	200	55	27.5
Sclerotia from soil samples taken two days after treatment, soil wet	197	45	22.8

to kill to a depth of 1 foot when allowed uninterrupted downward movement. A higher degree of effectiveness can probably not be expected under the circumstances. The rate of application used was equivalent to applications of 200 pounds per acre; greater dosages would probably be prohibitive in cost. Beside the high cost, the greatest handicap to practical application of chloropicrin soil treatment in open fields is the difficulty of providing complete confinement of the gas.

The Effectiveness of Chloropicrin Against Sclerotia in the Surface Layers of Piles of Screenings at a Beet-Receiving Station.—To eliminate the most important method of spreading *Sclerotium Rolfsii* and other parasites from one beet field to another, the screenings at the beet-receiving stations in California are piled on nonagricultural land instead of being returned to each grower's truck as was formerly done. As an additional precaution, destruction of sclerotia in such piles seemed desirable.

In October and November 1934, several attempts were made with chloropicrin to kill sclerotia planted in the surface material of piles of screenings accumulated at a beet-receiving station. In earlier experiments[6] the heat of fermentation in such piles had proved sufficient to kill

[6] Unpublished data.

sclerotia throughout the mass except for a comparatively thin zone at the surface, usually not more than a foot thick. The work described here was intended to kill the sclerotia that might be present in these surface zones.

The screenings are composed of variable amounts of soil from different fields and of leaves and the slender, broken taproots of the beets. On drying at 110° C for 48 hours, samples of such material, taken before the fall rains, show a moisture loss of 12 to 17 per cent. It was in newly made piles (not more than one to two days' accumulation) of such material that the experiments were conducted. The piles had been made with a dump truck, each load being deposited against the previous one, constituting a heap about 12 feet wide, 3 feet in extreme depth, and of indefinite length. For purposes of experiment about 15 feet of a pile was used. Even such loosely built, shallow piles heated readily, reaching 60° C in a day or two at a depth of 1 foot.

For convenience, test samples of 50 sclerotia were mixed with several grams of soil, and the mass tied in cheesecloth parcels to which tags on strings were attached. Such samples were distributed with uniform spacing at distances of several feet over the whole surface of several piles and were placed at depths of 12 inches, 9 inches, 6 inches, and 3 inches. Others were placed on the surface and just barely covered with screenings. At the time of placing the sclerotia, the temperature of the pile was recorded for the position of each test sample.

Chloropicrin was then injected into the pile with spacing of the points of application 1 foot each way. The dosage was 2½ cubic centimeters of chemical for each square foot of surface. Immediately after injection of the chloropicrin, the pile was covered with the glue-and-paraffin-treated kraft paper "chloropicrin proof" obtained from Dr. G. H. Godfrey. As before, the edges of the strips of paper were jointed by a strip of Scotch tape. At the periphery, the paper was secured by loading it with a continuous heap of soil. Again difficulty was experienced when the Scotch tape loosened in spots, partly as a result of moisture condensing on the lower side of the paper. Thus, too, a rather rapid escape of the gas took place, for there was no appreciable odor of chloropicrin about the surface of the pile when the test samples were removed 48 hours after the injection.

The sclerotia were removed from the cheesecloth, washed free of soil on a screen in the laboratory, and surface-sterilized for 45 seconds with 1:1,000 $HgCl_2$ before being plated on potato-dextrose agar. The results of culturing the sclerotia are presented in table 16, where the numbers of sclerotia tested in the various trials have all been combined for economy of space.

As will be noted, some few sclerotia escaped at a depth of 12 inches where the temperature might itself have been lethal (50° C killing sclerotia in water within 2 hours). On the whole, however, the figures show that killing, whether by high temperature, by the action of chloropicrin, or by both, was much more complete at the greater depth, up to 15 per cent of the test sclerotia surviving on the surface. It seems obvious that some means of making a tight cover is essential to such work.

The danger of disseminating sclerotia from piles of screenings to un-

TABLE 16

EFFECTIVENESS OF CHLOROPICRIN IN KILLING SCLEROTIA IN THE SURFACE LAYER OF PILES OF SCREENINGS AT A BEET-RECEIVING STATION

Depth of sclerotia, inches	Number of trials	Number of test lots	Sclerotia plated	Viable sclerotia	Per cent of sclerotia germinated	Temperature of screenings at position of test lots, degrees centigrade
Surface	2	26	1,300	193	14.80	35°
3	4	42	2,100	55	2.60	30°–55°
6	3	37	1,850	3	0.16	38°–55°
9	3	21	1,050	0	0.00	Unknown
12	3	34	1,700	7	0.41	55°–67°
Control	2	2	100	89	89.00	.....

infested fields has been removed by moving the screenings to locations that present no menace to cropped land. Thus the necessity for using fungicides is now avoided.

SUMMARY

Sclerotium Rolfsii, which causes southern sclerotium rot of sugar beets, has been found to grow on media having a wide range of hydrogen-ion concentration, rapid growth taking place up to pH 7.9. In the field the occurrence of rot on sugar beets is apparently not greatly affected by soils having values up to pH 8. Liming of soils, except with excessive amounts, has failed to reduce significantly infection of carrots in the laboratory; and in a single trial, moderate applications of lime to soils in the field reduced only slightly the amount of infection in a sugar-beet crop.

Seventeen water-soluble chemicals and fungicidal agents considered to have possibilities for use against soil fungi have been subjected to laboratory tests in vitro, and most of them also by application to soils. These are namely: acetic acid, ammonia, ammonium thiocyanate, boric acid, calcium cyanamid, chlorinated lime, carbon disulfide (water emulsion), calcium hydroxide, Dowicides B, H, and P, formaldehyde, mercuric chloride, phenol, potassium ethyl xanthate, pyroligneous acid, and sodium

hydroxide. None of these agents has proved more effective than formaldehyde under all conditions, though several, including ammonia, are more potent in vitro.

Solutions of formaldehyde, ammonia, and sodium hydroxide have been tested further in infested fields. With one exception, formalin (37 per cent formaldehyde) diluted 1:100 and applied at 3 gallons per square foot of soil surface killed sclerotia completely to a depth of 6 inches. Ammonia (28 per cent NH_3) and sodium hydroxide in the same strengths and at the same rates failed to reduce noticeably the numbers of viable sclerotia in the surface 6 inches.

Early removal of diseased beets from infested fields and chemical disinfestation of the surrounding soil reduced the occurrence of the disease on adjacent plants in the same row, but the effect of the treatment did not extend to the other rows. The number of viable sclerotia that remained to affect subsequent plantings was materially reduced.

The use of anhydrous ammonia by injection has been considered, but, even with almost air-dry soil, the absorptive capacity of the soil used in the experiment seems to preclude the possibility of thoroughly permeating soil in the field with ammonia.

Five volatile fungicides, insoluble in water, have been submitted to laboratory test. Xylene, tetrachlorethane, and pentachlorethane failed to kill sclerotia in doses as great as 5,000 parts per million of soil when the sclerotia were at least partially wet and the atmosphere saturated with water vapor; chloropicrin and carbon disulfide killed in doses as low as 100 and 600 parts per million of soil respectively. When properly confined, 2 cubic centimeters of chloropicrin per square foot of soil surface, injected at a depth of 6 inches, suffices to kill to a depth of 1 foot in the soil types considered.

Though the use of chloropicrin in the field has been attempted, the results secured in the single trial were unsatisfactory, no doubt both because of too small an application of the chemical and because of failure to confine the gas adequately.

When chloropicrin was used to kill sclerotia in the surface zone of piles of screenings at sugar-beet receiving stations, about 97 per cent of the sclerotia were killed; but it was difficult to confine the gas. Disposal of screenings in isolated spots has rendered treatment with fungicides unnecessary.

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FURTHER STUDIES ON THE INHERITANCE OF RESISTANCE TO POWDERY MILDEW OF BEANS

BJARNE DUNDAS

FURTHER STUDIES ON THE INHERITANCE OF RESISTANCE TO POWDERY MILDEW OF BEANS[1, 2]

BJARNE DUNDAS[3]

THIS PAPER PRESENTS the results from testing various crosses of beans (*Phaseolus vulgaris* L.) for resistance to powdery mildew (*Erysiphe polygoni* D. C.). These crosses were made between the resistant varieties Striped Hopi, Lady Washington, Hungarian, Yellow, *Phaseolus vulgaris* 5053, Long Kidney, Pinto, and Pink, and the susceptible varieties Robust, Small White, Kotenashi, and Red Kidney, and the semiresistant variety Long Roman.

METHODS

The method of testing by inoculating detached leaflets supported on a 10 per cent sucrose solution in petri dishes as described in an earlier publication[4] has also been used in this investigation. The culture of mildew was the same single-spore strain (now designated as strain 1) used in the work previously reported. The F_1 and F_2 plants were tested in the petri dishes, the F_3 for some crosses in petri dishes and for others by field inoculation with the same strain of the mildew. Readings of the severity of mildew are given on a scale of 0–4 for the dish tests as previously described. The field readings indicate only whether the plants are resistant or suspectible. No difference was observed among the resistant varieties, and all showed complete absence of mildew; but differences were observed among the susceptible varieties, and between the susceptible varieties as a group, the resistant varieties as a group, and the semiresistant variety.

The crosses were all made in the greenhouse and most of the progenies grown in the field and in the greenhouses at the California Agricultural Experiment Station at Berkeley during the years 1933–1936. The F_3 populations of *Phaseolus vulgaris* 5053 × Red Kidney, Long Roman × Yellow, Long Kidney × Red Kidney, *P. vulgaris* 5053 × Pinto, and Long Kidney × Pinto were grown in the field at the Associated Seed Growers' breeding grounds at Milpitas, California, in 1937.

[1] Received for publication June 26, 1939.

[2] The writer is indebted to W. W. Mackie, F. N. Briggs, M. W. Gardner, and C. E. Yarwood for their assistance. The experiment herein reported was completed on the grounds of the Associated Seed Growers, Inc., while the writer was employed there as a plant breeder, and acknowledgment is due for their coöperation.

[3] Former Research Assistant in the Division of Agronomy; resigned June 30, 1932.

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MILDEW REACTIONS OF THE PARENTAL MATERIAL

Table 1 gives readings of the severity of powdery mildew on the parental varieties based upon field infection and petri-dish inoculations.

The varieties Striped Hopi, Lady Washington, Hungarian, Yellow, *Phaseolus vulgaris* 5053, Long Kidney, and Pinto proved resistant in all dish tests. In 1932, 1933, 1935, and 1937, no mildew developed on them in the field at Berkeley, but in 1934 a small amount developed owing to the presence of a new physiologic form (form 3) of the mildew. When this

TABLE 1

MILDEW REACTION OF VARIETIES OF BEANS IN DISH TESTS AND IN THE FIELD

Variety	Accession No.	Number of plants with the reaction indicated								Rating*
		Dish readings						Field readings		
		0	*t*	1	2	3	4	Resistant	Susceptible	
Striped Hopi	4927	15	0	0	0	0	0	19	0	R
Lady Washington	84(213)32	15	0	0	0	0	0	20	0	R
Hungarian	4404	15	0	0	0	0	0	36	0	R
Yellow	4429	15	0	0	0	0	0	78	0	R
Phaseolus vulgaris	5053	40	0	0	0	0	0	40	0	R
Long Kidney	5045	15	0	0	0	0	0	40	0	R
Pinto	4369	16	0	0	0	0	0	38	0	R
Pink	4436	..	22	5	0	0	0	20	0	R
Robust	4458	..	0	0	0	8	7	0	38	S
Small White	4546	..	0	0	0	2	13	0	38	S
Kotenashi	4412	..	0	0	0	0	15	0	40	S
Red Kidney	4462	..	0	0	0	0	15	0	40	S
Long Roman	4521	..	0	0	0	15	0	0	40	SR

* R=resistant; S=susceptible; SR=semiresistant.

new form was artificially introduced in the field plots early in 1936, the infection became rather severe, but in other places where it was not introduced, the varieties above mentioned remained free from infection.

In dish tests, the Pink variety proved to be susecptible to form 1 in the youngest stages, but it soon became resistant, with only a slight development of mildew, readings being *t* and 1 even in rather old stages. In the field, it was resistant to form 1 but susceptible to the newly discovered form 3, as evidenced by a heavy natural infection in 1934.

The Long Roman variety was susceptible in dish tests, usually with a reading of 3. In the field in 1932 no infection was noticed before late in the fall after most varieties were harvested. In 1933, 1935, 1936, and 1937, a small amount of infection was present; but in 1934 the variety was more severely infected, as well as in 1936, when it was inoculated with form 3 of the fungus.

The Robust variety showed in 1932–1937 a light to medium-heavy field infection. It was highly susceptible in the dish tests, with readings of 3 and 4.

Small White, Kotenashi, and Red Kidney showed progressively heavier infection in field tests; all three varieties gave readings of 4 in all dish tests, with the exception of 2 readings of 3 for Small White.

TABLE 2

MILDEW REACTION OF THE F_1 AND F_2 PROGENIES FROM CROSSES BETWEEN SUSCEPTIBLE AND RESISTANT OR SEMIRESISTANT VARIETIES OF BEANS AND OF THE PARENT VARIETIES GROWN WITH THE F_2 POPULATION

Hybrid or variety	F_1 dish test	F_2								
		Grown*	Number of plants with a dish test of:						Ratio of resistant : susceptible	D/PE
			0	*t*	1	2	3	4		
Hybrids										
Robust × Striped Hopi	0, *t*	F	40	13	0	0	14	4	53 : 18	0.10
Striped Hopi × Robust	0	G	58	2	1	0	10	9	61 : 19	0.38
Lady Washington × Small White	0	F	11	1	4	0	3	2	16 : 5	0.19
Long Roman × Lady Washington	0	G	49	8	1	0	9	9	58 : 18	0.39
Robust × Hungarian	0, *t*	F	27	18	5	1	7	18	51 : 25	2.35
Long Roman × Hungarian	0	F	54	6	8	8	7	18	76 : 25	0.09
Hungarian × Long Roman	0	G	42	19	3	0	7	5	64 : 12	2.75
Yellow × Long Roman	0	F	25	26	6	9	12	11	66 : 23	0.27
Robust × Yellow	0	G	38	9	0	0	3	14	47 : 17	0.43
Phaseolus vulgaris 5053 × Red Kidney	*t*	F	59	10	2	1	18	6	72 : 24	0.00
Long Kidney × Red Kidney	1	F	19	27	27	21	21	7	94 : 28	0.46
Robust × Pink	1	F	14	18	13	15	9	14	60 : 23	0.85
	1	G	0	40	24	16	0	22	80 : 22	0.51
Pink × Kotenashi	1	G	1	35	20	4	8	16	60 : 24	1.12
Parent varieties										
Striped Hopi	..	..	15	0	0	0	0	0	....	....
Lady Washington	..	..	15	0	0	0	0	0	....	....
Hungarian	..	..	15	0	0	0	0	0	....	....
Yellow	..	..	15	0	0	0	0	0	....	....
Long Kidney	..	..	9	0	0	0	0	0	....	....
Phaseolus vulgaris 5053	..	..	5	1	0	0	0	0	....	....
Pink	..	..	0	9	3	0	0	0	....	....
Small White	..	..	0	0	0	0	2	13	....	....
Robust	..	..	0	0	0	0	13	14	....	....
Red Kidney	..	..	0	0	0	0	6	9	....	....
Kotenashi	..	..	0	0	0	0	0	9	....	....

* F = field-grown, G = greenhouse-grown.

ROBUST (S) × STRIPED HOPI (R), AND STRIPED HOPI × ROBUST

The F_1 of Robust (S) × Striped Hopi (R) gave 1 reading of 0 and 1 of *t*, (table 2). The reading of the reciprocal cross was 0. The F_2 generations of the reciprocal crosses gave practically identical results: the Robust × Striped Hopi cross had 53 resistant and 18 susceptible plants, and its reciprocal, 61 resistant and 19 susceptible. No plants had a reading of 2 and only 1 a reading of 1; thus there is a very clear distinction between the resistant and susceptible plants. The ratios for both crosses give a very close fit to a 3:1 ratio and indicate that the resistance in Striped Hopi is controlled by a single dominant Mendelian factor pair.

TABLE 3

Mildew Reaction of F_3 Progenies of Robust (S) × Striped Hopi (R) Beans*

Dish test of F_2 parent	F_3			Families in F_2 groups	Dish test of F_2 parent	F_3			Families in F_2 groups
	Resistant plants	Susceptible plants	$\frac{D}{PE}$			Resistant plants	Susceptible plants	$\frac{D}{PE}$	
t	54	15	0.93	21 heterozygous resistant					
t	38	14	0.47		4	0	37	..	10 homozygous susceptible
t	62	14	1.96		4	0	58	..	
t	72	20	1.07		3	0	33	..	
t	24	8	0.00		3	0	45	..	
t	18	7	0.51		3	0	76	..	
t	25	6	1.07		3	0	32	..	
0	28	13	1.47		3	0	36	..	
0	23	7	0.31		3	0	43	..	
0	33	7	1.62		3	0	30	..	
0	26	7	0.76		3	0	46	..	
0	22	5	1.15						
0	75	21	1.05		*t*	37	0	.	9 homozygous resistant
0	53	17	0.20		*t*	56	0	..	
0	30	8	0.83		0	49	0	..	
0	25	11	1.14		0	40	0	..	
0	45	13	0.68		0	64	0	..	
0	84	26	0.49		0	40	0	..	
0	22	7	0.16		0	44	0	..	
0	28	8	0.57		0	57	0	..	
0	24	6	0.94		0	41	0	..	

* Mildew reaction of parents grown at the same time: Robust, 0 resistant, 45 susceptible plants. Striped Hopi, 32 resistant, 0 susceptible plants.

The Robust × Striped Hopi cross was carried to the F_3 generation (table 3). The 10 families from susceptible F_2 plants with the readings of 3 and 4 contained only susceptible progeny. Of the 30 families from resistant F_2 plants, 21 segregated in a 3:1 ratio and 9 were homozygous for resistance. The 2:1 ratio of segregating: homozygous families is in accordance with expectations on the basis of a one-factor difference for

resistance between Robust and Striped Hopi and confirms the results obtained in the F_2. It should be noted that the F_2 dish readings of 0 and *t* did not give any hint as to homozygosity or heterozygosity for resistance.

LADY WASHINGTON (R) × SMALL WHITE (S), AND LONG ROMAN (SR) × LADY WASHINGTON (R)

In both Lady Washington (R) × Small White (S), and Long Roman (SR) × Lady Washington (R), the F_1 plants gave a 0 mildew reaction

TABLE 4

MILDEW REACTION OF F_3 PROGENIES OF LADY WASHINGTON (R) × SMALL WHITE (S) BEANS*

Dish test of F_2 parent	F_3			Families in F_2 groups	Dish test of F_2 parent	F_3			Families in F_2 groups
	Resistant plants	Susceptible plants	D / PE			Resistant plants	Susceptible plants	D / PE	
					4	0	13	..	5 homozygous susceptible
					4	0	45	..	
1	41	8	2.08	10 heterozygous resistant	3	0	18	..	
1	25	6	1.07		3	0	39	..	
1	40	8	2.02		3	0	18	..	
1	25	9	0.29						
t	26	6	1.21		0	27	0	..	6 homozygous resistant
0	25	5	1.56		0	37	0	..	
0	20	5	0.86		0	42	0	..	
0	36	5	2.81		0	40	0	..	
0	42	7	2.57		0	25	0	..	
0	25	7	0.61		0	33	0	..	

* Mildew reaction of parents grown at the same time: Lady Washington, 20 resistant, 0 susceptible plants. Small White, 0 resistant, 26 susceptible plants.

(table 2), and the distributions of the F_2 readings were similar, with a distinct separation between resistant and susceptible plants by the absence of class 2. The ratios for both crosses gave a close fit to a 3:1 ratio, and indicate that the resistance in Lady Washington is due to a single dominant Mendelian factor.

The Lady Washington × Small White cross was carried to the F_3 generation. The F_2 population of this cross was small, but every plant had a fair yield of seed and all were included in the F_3 generation (see table 4). The susceptible F_2 plants with readings of 3 or 4 gave only susceptible progenies. The 4 F_2 plants with a reading of 1 and the 1 plant with a reading of *t* proved to be heterozygous for resistance, while the 11 with readings of 0 were both homozygous and heterozygous. The F_3 generation thus had 5 susceptible families, 10 heterozygous families segregating in a 3:1 ratio, and 6 families homozygous for resistance, which is very

close to the expected 1:2:1 ratio, and confirms the conclusion drawn from the F_2 results, that is, that the resistance in Lady Washington is due to a single dominant Mendelian factor.

ROBUST (S) × HUNGARIAN (R), LONG ROMAN (SR) × HUNGARIAN (R), AND HUNGARIAN (R) × LONG ROMAN (SR)

The F_1 of Robust (S) × Hungarian (R) gave 1 reading of 0 (table 2) and 1 of *t;* Long Roman (SR) × Hungarian (R) and the reciprocal cross had 0 in both tests.

In the F_2 (table 2), the Robust × Hungarian cross had 1 reading in

TABLE 5

Mildew Reaction of the F_3 Progenies of Robust (S) × Hungarian (R) Beans*

Dish test of F_2 parent	F_2			Families in F_2 groups	Dish test of F_2 parent	F_3			Families in F_2 groups
	Resistant plants	Susceptible plants	D/PE			Resistant plants	Susceptible plants	D/PE	
					4	0	30	..	10 homozygous susceptible
1	11	3	0.46	19 heterozygous resistant	4	0	24	..	
1	36	11	0.38		4	0	51	..	
t	33	15	1.49		4	0	33	..	
t	32	13	0.89		4	0	34	..	
t	22	10	1.21		4	0	16	..	
t	12	7	1.77		4	0	63	..	
t	23	11	1.47		3	0	50	..	
t	18	4	1.09		3	0	52	..	
t	13	7	1.53		3	0	45	..	
t	13	6	0.98						
0	20	5	0.86		2	50	0	..	11 homozygous resistant
0	23	6	0.80		1	48	0	..	
0	29	5	2.06		1	50	0	..	
0	19	6	0.17		*t*	31	0	..	
0	12	4	0.00		*t*	17	0	..	
0	71	22	0.44		*t*	51	0	..	
0	18	4	1.09		0	34	0	..	
0	21	7	0.00		0	34	0	..	
0	31	7	1.39		0	14	0	..	
					0	36	0	..	
					0	23	0	..	

* Mildew reaction of parents grown at the same time: Robust, 0 resistant, 39 susceptible plants. Hungarian, 26 resistant, 0 susceptible plants.

the intermediate class 2, the Long Roman × Hungarian cross had 8, and the reciprocal of the latter had 0 in that class. The difference between the reciprocal crosses is attributed mainly to the difference in growing conditions, one having been grown in the greenhouse, the other in the field. A similar difference was found between a greenhouse- and field-grown

F_2 population of Long Roman × Pinto,[5] the field-grown population in both cases giving more numerous intermediate readings.

The plant in the intermediate class 2 proved to be homozygous for resistance in the case of Robust × Hungarian, as will be seen from an examination of its F_3 progeny (table 5). If the 8 class-2 plants in Long Roman × Hungarian are assumed to be resistant, all the data agree with the 3:1 ratio, which indicates that the resistance of Hungarian is due to a single dominant factor.

In the Robust × Hungarian cross (table 5), the 10 families from susceptible F_2 plants with readings of 3 and 4 produced only susceptible F_3 plants. Of the 30 families from resistant F_2 plants, 19 were heterozygous for resistance, segregating in a 3:1 ratio, and 11 homozygous for resistance. This is close to the expected 2:1 ratio and confirms the F_2 findings that the resistance in Hungarian is due to a single dominant Mendelian factor.

ROBUST (S) × YELLOW (R) AND YELLOW (R) × LONG ROMAN (SR)

The F_1 plants in Robust (S) × Yellow (R) and Yellow (R) × Long Roman (SR) (table 2) gave only readings of 0 like the resistant parent.

In the F_2 population of the Robust × Yellow cross, there is a sharp distinction between the resistant and susceptible plants marked by the absence of readings in classes 1 and 2, while the Yellow × Long Roman cross, with a field-grown population, had 6 and 9 respectively in classes 1 and 2. This difference is not entirely due to the fact that one population was grown in the field and the other in the greenhouse. The Long Roman in its crosses tends to level out the sharp difference found between resistant and susceptible plants in other crosses. The Long Roman variety itself has a certain resistance in the field, while in the dishes it is susceptible, usually giving a reading of 3. If class 2 is counted with the resistants, as previously, both the Yellow crosses have a very close fit to a 3:1 ratio, and Yellow must, like the preceding resistant varieties, owe its resistance to a single dominant Mendelian factor.

An F_3 population of Yellow × Long Roman was grown from seed from 10 susceptible and 33 resistant F_2 plants, including 4 with a mildew reading of 2 (table 6). The F_2 plants were the progenies from one F_1 plant. The populations of the 10 susceptible F_3 families ranged from 14 to 27, the susceptible plants usually giving less seed because of mildew attack in the field. The populations of the 33 resistant F_3 families ranged from 18 to 50 with only 5 families below 27, the maximum population of the susceptible families.

[5] Dundas, Bjarne. Inheritance of resistance to powdery mildew in beans. Hilgardia 10(8):250. 1936.

TABLE 6

Mildew Reaction of the F_3 Progenies of Yellow (R) × Long Roman (SR) Beans*

Dish test of F_2 parent	F_3								Families in F_2 groups
	Number of plants with a dish test of:						Ratio of resistant : susceptible	D / PE	
	0	*t*	1	2	3	4			
4	0	0	0	0	13	14	0 : 27	....	
4	0	0	0	0	7	11	0 : 18	....	
4	0	0	0	0	6	8	0 : 14	....	
4	0	0	0	0	7	12	0 : 19	....	
3	0	0	0	0	12	15	0 : 27	....	10 homozygous susceptible
3	0	0	0	0	12	10	0 : 22	....	
3	0	0	0	0	10	10	0 : 20	....	
3	0	0	0	0	1	17	0 : 18	....	
3	0	0	0	0	4	20	0 : 24	....	
3	0	0	0	0	10	5	0 : 15	....	
2	3	18	4	0	8	0	25 : 8	0.15	
2	16	10	1	1	5	1	28 : 6	1.47	
2	5	13	3	1	8	0	22 : 8	0.31	
2	18	12	1	0	7	3	31 : 10	0.13	
1	6	8	0	0	3	2	14 : 5	0.59	
1	20	9	2	0	5	5	31 : 10	0.13	
1	12	23	3	0	4	4	38 : 8	1.26	
1	7	18	2	1	6	5	28 : 11	0.69	
t	10	12	1	0	4	2	23 : 6	0.80	
t	16	14	0	0	5	3	30 : 8	0.83	21 heterozygous resistant
t	5	9	0	0	4	0	14 : 4	0.40	
t	14	20	3	0	8	5	37 : 13	0.24	
t	12	14	0	0	6	4	26 : 10	0.57	
t	13	15	1	1	9	0	30 : 9	0.41	
t	8	21	1	0	7	1	30 : 8	0.83	
t	5	14	0	0	6	0	19 : 6	0.17	
0	12	19	0	0	9	0	31 : 9	0.54	
0	6	20	3	0	9	2	29 : 11	0.54	
0	20	14	0	0	10	0	34 : 10	0.52	
0	7	10	1	0	5	1	18 : 6	0.00	
0	11	14	1	0	5	5	26 : 10	0.57	
1	31	8	1	0	0	0	40 : 0	....	
1	25	10	4	0	0	0	39 : 0	....	
t	10	22	2	0	0	0	34 : 0	....	
t	18	15	1	0	0	0	34 : 0	....	
t	20	7	2	0	0	0	29 : 0	....	
t	11	10	2	0	0	0	23 : 0	....	12 homozygous resistant
t	23	6	0	0	0	0	29 : 0	....	
0	28	4	3	0	0	0	35 : 0	....	
0	27	13	1	0	0	0	41 : 0	....	
0	18	10	0	0	0	0	28 : 0	....	
0	28	17	0	0	0	0	45 : 0	....	
0	31	7	0	0	0	0	38 : 0	....	

* Mildew reaction of parents grown at the same time: Yellow, all 15 plants grown, dish-test reading of 0. Long Roman, all 15 plants grown, dish-test reading of 3.

Of the 33 resistant F_2 plants (readings 0, *t*, 1, and 2), 12 proved to be homozygous and 21 heterozygous for resistance, which is close to the 1:2 ratio expected from a random sample. The 21 heterozygous F_2 plants segregated in F_3 in accordance with the single-factor hypothesis. The

TABLE 7

MILDEW REACTION OF THE F_3 PROGENIES OF PHASEOLUS VULGARIS 5053 (R) × RED KIDNEY (S) BEANS*

Dish test of F_2 parent	F_3 Number of plants with a dish test of: 0	*t*	1	2	3 and 4	Ratio of resistant : susceptible	D/PE	Families in F_2 groups
3	0	0	0	0	23	0 : 23	....	9 homozygous susceptible
3	0	0	0	0	28	0 : 28	....	
3	0	0	0	0	19	0 : 19	....	
3	0	0	0	0	23	0 : 23	....	
3	0	0	0	0	26	0 : 26	....	
3	0	0	0	0	25	0 : 25	....	
3	0	0	0	0	15	0 : 15	....	
3	0	0	0	0	18	0 : 18	....	
3	0	0	0	0	15	0 : 15	....	
t	15	0	0	0	4	15 : 4	0.59	17 heterozygous resistant
t	21	0	0	0	5	21 : 5	1.00	
t	20	3	0	0	4	23 : 4	1.81	
t	27	3	0	0	11	30 : 11	0.40	
0	12	3	1	0	6	16 : 6	0.36	
0	12	3	0	0	5	15 : 5	0.00	
0	10	5	2	0	4	17 : 4	0.93	
0	14	2	0	0	4	16 : 4	0.76	
0	24	0	0	0	8	24 : 8	0.00	
0	20	0	0	0	6	20 : 6	0.34	
0	16	0	0	0	4	16 : 4	0.76	
0	9	3	0	0	7	12 : 7	1.31	
0	14	0	0	0	6	14 : 6	0.76	
0	20	4	2	1	7	27 : 7	0.88	
0	24	5	0	0	5	29 : 5	2.06	
0	31	2	1	0	7	34 : 7	1.74	
0	20	6	0	1	9	27 : 9	0.00	
0	29	0	0	0	0	29 : 0	....	8 homozygous resistant
0	16	2	1	1	0	20 : 0	....	
0	26	0	0	0	0	26 : 0	....	
0	21	0	0	0	0	21 : 0	....	
0	24	0	0	0	0	24 : 0	....	
0	32	0	0	0	0	32 : 0	....	
0	20	0	0	0	0	20 : 0	....	
0	20	0	0	0	0	20 : 0	....	

* Mildew reaction of parents grown at the same time: *Phaseolus vulgaris* 5053, all 26 plants grown, dish-test reading of 0. Red Kidney, all 20 plants grown, dish-test reading of 3.

susceptible F_2 plants (readings 3 and 4) gave only susceptible progeny. It should be noted that the tested F_2 plants with a reading of 2 were all heterozygous for resistance, while readings 1, *t*, and 0 did not give any indication of a homozygous or heterozygous condition.

PHASEOLUS VULGARIS 5053 (R) × RED KIDNEY (S)

The F_1 plant of the *Phaseolus vulgaris* 5053 (R) × Red Kidney (S) cross gave a reading of *t* in the dish test, which indicates that the resistance is dominant (table 2). The F_2 generation all came from one plant and consisted of 72 resistant and 24 susceptible plants. This is a perfect 3 : 1 ratio and indicates a single dominant factor for resistance to mildew in *P. vulgaris* 5053. All the plants that gave enough seed were used for the F_3 generation (table 7). The 2 plants with a reading of 1, the 1 with a reading of 2, and the 6 with a reading of 4 had not enough seed for an F_3 population. The populations of the F_3 families ranged between 15 and 41 and were dish-tested as indicated in table 7. No distinction was made between the susceptible readings 3 and 4, which are recorded together under one heading.

Of the 25 resistant F_2 plants, 8 were homozygous and 17 heterozygous for resistance.This is close to the 1 : 2 ratio expected on the basis of a single factor for resistance. The 17 heterozygous F_2 plants segregated in F_3 according to expectations. All F_2 plants with a reading of *t* were heterozygous for resistance while those with a reading of 0 were both homozygous and heterozygous for resistance.

LONG KIDNEY (R) × RED KIDNEY (S)

The F_1 of Long Kidney (R) × Red Kidney (S) gave a reading of 1 in the dish test (table 2), which indicates the dominance of the resistance in Long Kidney.

The F_2 population of this cross has a proportionately much larger number of plants with an intermediate reading than did that of any other cross ; as shown in table 2, out of a total population of 122, there are 27 with a reading of 1 and 21 with a reading of 2. From 7 of the F_2 plants with readings of 2 there were grown F_3 populations (table 8), all of which segregated, which shows that these plants were heterozygous for resistance. Assuming that the remainder of this class (reading of 2) of F_2 plants are also resistant, there are 94 resistant and 28 susceptible plants, which agrees fairly closely with the 3 : 1 ratio and indicates that the resistance of Long Kidney is due to a single dominant factor.

Only 25 plants had sufficent seed for F_3 populations. These were all planted and gave F_3 families with populations ranging from 20–49 (table 8). The 4 families from susceptible F_2 plants gave all susceptible plants. Of the 21 families from the resistant plants, 14 were heterozygous for resistance, segregating in a 3 : 1 ratio, and 7 homozygous for resistance. This is a perfect 2 : 1 ratio and confirms the F_2 findings that the resistance in the Long Kidney is due to a single dominant factor. It should be

noted that all the progeny-tested F_2 plants with readings of 2 were heterozygous for resistance while those with readings of 0, *t*, and 1 were both homozygous and heterozygous.

PINK (R) × KOTENASHI (S) AND ROBUST (S) × PINK (R)

The F_1 in Pink (R) × Kotenashi (S) and Robust (S) × Pink (R) all gave a reading of 1 when leaves were taken from older plants (table 2). In the early seedling stage the F_1 plants gave a susceptible reaction of 3

TABLE 8

MILDEW REACTION OF THE F_3 PROGENIES OF LONG KIDNEY (R) × RED KIDNEY (S) BEANS*

Dish test of F_2 parent	F_3			Families in F_2 groups	Dish test of F_2 parent	F_3			Families in F_2 groups
	Resistant plants	Susceptible plants	D / PE			Resistant plants	Susceptible plants	D / PE	
2	18	6	0.00	14 heterozygous resistant					
2	18	5	0.53		4	0	24	..	4 homozygous susceptible
2	36	13	0.37		4	0	24	..	
2	23	7	1.25		4	0	20	..	
2	23	8	0.15		3	0	26	..	
2	21	8	0.48						
2	18	6	0.00		1	28	0	..	7 homozygous resistant
1	26	9	0.14		1	23	0	..	
1	31	9	0.54		*t*	26	0	..	
1	16	5	0.19		*t*	30	0	..	
t	23	7	0.31		0	30	0	..	
t	25	9	0.26		0	31	0	..	
0	21	7	0.00		0	20	0	..	
0	22	6	0.65						

* Mildew reaction of parents grown at the same time: Red Kidney, 0 resistant, 20 susceptible. Long Kidney, 20 resistant, 0 susceptible.

or 4. This agrees with earlier findings[6] that the Pink variety was susceptible in the early seedling stage but resistant later.

The F_2 generation of Pink × Kotenashi was grown in the greenhouse. In an early test, most plants appeared rather susceptible. New tests were made when the plants were approximately one and two months old, and in the last test three leaflets from each plant were used. The average readings from the old leaflets of the two last tests gave 60 resistant and 24 susceptible plants, not far from a ratio of 3:1 as in the previous crosses. This indicates that the resistance in Pink is also due to a single dominant factor pair.

An F_2 of Robust × Pink was also grown in the greenhouse. Five tests were made of this cross, the first when the plants were about 9–14 days old, the following 16, 24, 32, and 45 days later. The first test showed many

[6] Dundas, Bjarne. Inheritance of resistance to powdery mildew in beans. Hilgardia 10(8):246. 1936.

TABLE 9

MILDEW REACTION OF THE F_2 PROGENIES OF ROBUST (S) × PINK (R) BEANS*

Dish test of F_2 parent	Number of plants with a dish test of:						Ratio of resistant : susceptible	$\frac{D}{PE}$	Families in F_2 groups
	0	*t*	1	2	3	4			
4	0	0	0	0	25	8	0 : 33	....	
4	0	0	0	0	30	3	0 : 33	....	
4	0	0	0	0	28	0	0 : 28	...	
4	0	0	0	0	18	15	0 : 33	....	
4	0	0	0	0	15	6	0 : 21	....	
4	0	0	0	0	12	22	0 : 34	....	13
4	0	0	0	0	17	15	0 : 32	....	homozygous
4	0	0	0	0	31	11	0 : 42	....	susceptible
4	0	0	0	0	10	15	0 : 25	....	
4	0	0	0	0	29	12	0 : 41	....	
3	0	0	0	0	16	14	0 : 30	. .	
3	0	0	0	0	22	10	0 : 32	....	
3	0	0	0	0	33	0	0 : 33	....	
2	0	6	14	1	0	5	21 : 5	1.00	
2	2	20	20	1	10	3	43 : 13	0.50	
2	0	9	11	4	5	3	24 : 8	0.00	
2	1	9	13	0	4	4	23 : 8	0.15	
2	4	14	13	0	7	2	31 : 9	0.54	
2	0	12	14	0	4	2	26 : 6	1.23	
2	3	14	6	0	8	5	23 : 13	0.76	
2	0	8	13	2	3	3	23 : 6	0.80	
1	0	16	12	2	8	3	30 : 11	0.40	
1	0	9	15	3	5	5	27 : 10	0.42	
1	1	16	17	0	3	3	34 : 6	2.16	24
1	0	14	15	0	3	8	29 : 11	0.54	heterozygous
1	0	20	7	0	8	1	27 : 9	0.00	resistant
1	3	16	8	0	5	5	27 : 10	0.42	
1	5	13	10	1	5	5	29 : 10	0.14	
t	0	12	13	2	5	6	27 : 11	0.83	
t	2	12	9	0	4	5	23 : 9	0.61	
t	0	8	9	1	5	2	18 : 7	0.51	
t	0	16	3	0	3	4	19 : 7	0.34	
t	10	9	8	0	6	4	27 : 10	0.42	
t	0	7	16	2	5	2	25 : 7	0.61	
0	3	8	10	0	2	3	21 : 5	1.00	
0	7	10	3	0	5	2	20 : 7	0.16	
0	0	9	9	0	4	1	18 : 5	0.54	
1	0	17	15	0	0	0	32 : 0	....	
t	4	28	10	0	0	0	42 : 0	....	
t	2	15	6	0	0	0	23 : 0	....	
t	3	18	5	0	0	0	26 : 0	....	
t	8	9	19	2	0	0	38 : 0	....	
t	3	12	8	0	0	0	23 : 0	....	12 homozygous
0	8	12	2	0	0	0	22 : 0	....	resistant
0	5	16	12	0	0	0	33 : 0	. . .	
0	0	23	10	0	0	0	33 : 0	....	
0	2	22	15	0	0	0	39 : 0	. . .	
0	3	12	12	0	0	0	27 : 0	....	
0	3	18	8	0	0	0	29 : 0	....	

* Mildew reaction of parents grown at the same time: Robust, 10 plants with a dish-test reading of 3, 5 with a reading of 4. Pink, 13 plants with a dish-test reading of 0, 2 with a reading of *t*.

plants to be susceptible which later became resistant. The results given in table 2 are based on the average of the later tests made on fully developed leaflets when the plants had become resistant. The fully developed leaflets usually show more resistance than the younger ones. The ratio 80 resistant : 22 susceptible, is fairly close $\left(\frac{D}{PE} = 0.51\right)$ to a 3 :1 ratio and confirms the finding of the Pink × Kotenashi cross.

Another F_2 population of Robust × Pink was grown in the field for the purpose of obtaining seed for an F_3 population. Only a single dish test was made of this when the plants had attained resistance, except for doubtful cases, which were retested. The result is seen from table 2 to be 60 resistant : 23 susceptible, close to the ratio obtained in the greenhouse-grown cross. The F_3 grown from this cross (table 9) was taken from 13 of the field-grown susceptible F_2 plants with readings of 3 and 4 and 36 of the resistant plants with readings of 0–2. The families from the 13 susceptible plants all gave susceptible progenies, while, of those from the resistant plants, 24 segregated in a ratio of 3 resistant : 1 susceptible and 12 were homozygous for resistance. The 2 :1 ratio of segregating : homozygous resistant families is in accordance with expectations on the basis of a one-factor difference for resistance between Robust and Pink and confirms the results obtained in the F_2. It should be noted that all the F_2 plants with readings of 2 and 1 (except 1 with a reading of 1) proved to be heterozygous for resistance, whereas the readings of 0 and *t* did not give any indication of homozygosity or heterozygosity of the plants.

CROSSES BETWEEN RESISTANT VARIETIES

Crosses between resistant varieties were made as follows and carried to the F_3 generation : Lady Washington × Pinto, Hungarian × Pinto, Yellow × Pinto, *Phaseolus vulgaris* 5053 × Pinto, Pinto × Striped Hopi, Long Kidney × Pinto, and Pinto × Pink. Crosses of Yellow × Hungarian and Yellow × *P. vulgaris* 5053 were carried to F_2. These were all similar in reaction to mildew and will be treated together. The F_1 plants gave only readings of 0, except the Yellow × Pinto cross which gave *t* for 1 of the 2 readings (see table 10).

The F_2 plants of all crosses (table 10) reacted much like the parents. The Lady Washington × Pinto cross had only 0 readings, as did the Yellow × Pinto, the Yellow × *Phaseolus vulgaris* 5053, *P. vulgaris* 5053 × Pinto, Pinto × Striped Hopi, and Long Kidney × Pinto crosses. The Hungarian × Pinto cross had 99 readings of 0 and 1 of *t*. The parents of this cross had readings of 0, but if a larger number of plants had been tested, the chances are that a reading of *t* might have occurred. The Yellow × Hungarian cross gave 82 readings of 0 and 9 of *t*. This population was

grown in the field, and, as observed before, the field-grown plants often show some higher readings than those grown in the greenhouse. Two populations of Pinto × Pink crosses gave the same proportion of the same readings; combined they gave 82 readings of 0, 43 of *t*, 12 of 1, and 8 of 2. These numerous intermediate readings, like those obtained with the Robust × Pink cross, indicate the presence of modifying factors.

The results of crosses with susceptible varieties (tables 2–9), pre-

TABLE 10

Mildew Reaction of the F_1, F_2, and F_3 Progenies of Certain Mildew-Resistant Bean Varieties

Hybrid and variety	F_1 dish test	F_2 and parents							F_3			
		Grown*	Number of plants with a dish test of:						Grown*	Number of families		Numerical range of populations
			0	*t*	1	2	3	4		Tested	Resistant	
Lady Washington × Pinto	0	G	93	0	0	0	0	0	F	48	48	22–84
Hungarian × Pinto	0	G	99	1	0	0	0	0	F	48	48	17–55
Yellow × Pinto	0 & *t*	G	97	0	0	0	0	0	F	45	45	17–58
Yellow × Hungarian	0	F	82	9	0	0	0	0	..	..	..	....
Yellow × *Phaseolus vulgaris* 5053	0	F	40	0	0	0	0	0	..	..	..	....
P. vulgaris 5053 × Pinto	0	F	136	0	0	0	0	0	F	47	47	18–43
Pinto × Striped Hopi	0	G	103	0	0	0	0	0	F	48	48	19–56
Long Kidney × Pinto	0	F	96	0	0	0	0	0	F	38	38	18–44
Pinto × Pink	0	F	43	21	6	5	0	0	..	..	..	....
Pinto × Pink	0	G	39	22	6	3	0	0	G	64	64	10–128

* F = field-grown; G = greenhouse-grown.

viously discussed, have shown that these resistant varieties, Pinto, Lady Washington, Hungarian, Yellow, Long Kidney, Striped Hopi, *Phaseolus vulgaris* 5053, and Pink, contain one dominant factor for resistance. The fact that intercrossing of these varieties gave no segregating plants indicates that they all contain the same main factor for resistance. Additional F_2 populations of all the crosses were grown in the field in order to obtain seed for F_3 generations. The individual plants were examined for mildew, but none were found to be infected.

F_3 generations of six of these crosses were grown in the field and one of these—Pinto × Pink—was also grown in the greenhouse. This one and also *Phaseolus vulgaris* 5053 × Pinto and Long Kidney × Pinto were dish-tested; the results on the others were taken from field readings. The

number of families found resistant and the range in the F_3 populations are given in table 10. None of the plants from any of the crosses showed any susceptibility, all families appeared to be homozygous for resistance. Thus the result from the F_2 is confirmed, namely, that the resistant varieties, Pinto, Lady Washington, Hungarian, Yellow, *Phaseolus vulgaris* 5053, Striped Hopi, Long Kidney, and Pink contain the same genetic factor for resistance.

SUMMARY

In determining the susceptibility of the F_1, F_2, and F_3 of crosses between resistant and susceptible varieties of beans (*Phaseolus vulgaris* L.) to powdery mildew (*Erysiphe polygoni* D. C.) (form 1), the mildew was grown in petri dishes on detached bean leaflets supported on cotton soaked in a 10 per cent sucrose solution as in earlier work. For certain F_3 generations, field inoculation was used. The susceptibility of individual plants was determined by inoculating detached leaflets or fragments of leaflets in petri dishes. Mildew readings were made on a scale of 0–4; where field inoculation was used, readings indicated only whether the plants were susceptible or resistant.

The varieties Striped Hopi, Lady Washington, Hungarian, Yellow, Long Kidney, *Phaseolus vulgaris* 5053, and Pinto were found to be resistant in all stages of development, while Pink was susceptible in its youngest stages but resistant in older stages. The varieties Robust, Small White, Kotenashi, and Red Kidney were susceptible, while Long Roman was semiresistant in the field but susceptible in the dishes.

The F_1 and F_2 of Robust (S) × Striped Hopi (R) and its reciprocal cross were tested by the dish method, and the F_3 of the former was tested by field inoculation. The F_2 segregated in a ratio of 3 resistant to 1 susceptible, both on the basis of the test of the individual F_2 plants and their F_3 progenies. This establishes the resistance to mildew to be due to a single dominant Mendelian factor pair.

Similar results were arrived at in crosses of Lady Washington × Small White, Long Roman × Lady Washington, Robust × Hungarian, Long Roman × Hungarian and its reciprocal cross, Yellow × Long Roman, Robust × Yellow, *Phaseolus vulgaris* 5053 × Red Kidney, Long Kidney × Red Kidney, Robust × Pink, and Pink × Kotenashi.

In crosses between resistant varieties, F_1, F_2, and F_3 from Lady Washington × Pinto, Hungarian × Pinto, Yellow × Pinto, *Phaseolus vulgaris* 5053 × Pinto, Pinto × Striped Hopi, Long Kidney × Pinto, Pinto × Pink, and F_1 and F_2 from Yellow × Hungarian and Yellow × *P. vulgaris* 5053 were all resistant, which indicates that these resistant varieties carry the same single Mendelian factor for resistance to mildew.

SNAPDRAGON RUST-RESISTANCE TRIALS 1937–1938

C. O. BLODGETT AND G. A. L. MEHLQUIST

SNAPDRAGON RUST-RESISTANCE TRIALS 1937–1938[1]

C. O. BLODGETT[2] AND G. A. L. MEHLQUIST[3]

INTRODUCTION

THROUGH THE EFFORTS of a number of workers, especially of Mains[4] and Emsweller and Jones,[5] it appeared in 1934 and 1935 as though the serious problem of rust on snapdragons would soon be solved. Some valuable commercial strains[6] had been released to the seed trade in 1931 and 1932, and development of others was proceeding at a rapid pace. Mains had warned of the possibility of the occurrence of physiologic forms[7] in the *Antirrhinum rust* (*Puccinia antirrhini* D. and H.), but when in 1936 the so-called "resistant" snapdragons began to show severe symptoms of rust, especially in the Salinas Valley, the question was raised by commercial seed growers as to whether this was due to a "breakdown" in the resistance resulting from breeding and cultural practices. Yarwood,[8] however, clearly demonstrated that the susceptibility of the resistant strains was due to the presence of one or more different forms of the rust, which evidently had not been prevalent before that time in the district where the earlier work had been done.

The purpose of this paper is to place on record the results of trials conducted during the seasons of 1937 and 1938, in an attempt to locate species, varieties, or strains of *Antirrhinum* immune, or at least highly resistant, to new as well as old forms of rust. It was hoped that, if such an *Antirrhinum* strain could be found, it might be used in the breeding of resistant or immune types suitable for floriculture and ornamental gardening.

This work is in part a continuation of that started by Emsweller and Jones. During the first season, 1936–37, the work was conducted through

[1] Received for publication June 30, 1939.

[2] Research Assistant in Genetics; resigned June 30, 1939.

[3] Instructor in Floriculture and Junior Floriculturist in the Experiment Station.

[4] Mains, E. B. Rust resistance in *Antirrhinum*. Phytopathology 25(11):977–91. 1935.

[5] Emsweller, S. L., and H. A. Jones. The inheritance of resistance to rust in the snapdragon. Hilgardia 8(7):197–211. 1934.

[6] Throughout this paper the word "strains" is used in the sense that it includes a smaller category of snapdragons than either "species" or "varieties"—that is, there were in certain cases in our trials, several strains of certain varieties of commercial snapdragon.

[7] In speaking of rusts, "forms" will be used to designate the different "races" or "physiologic forms."

[8] Yarwood, Cecil E. Physiologic races of snapdragon rust. Phytopathology 27(1): 113–15. 1937.

the Division of Truck Crops at the University Farm at Davis. In 1937, the work was transferred to the Division of Genetics at Berkeley, and it has been continued with Berkeley as headquarters.

MATERIALS AND METHODS

For these trials, seeds of about one hundred and forty samples of different species and strains of *Antirrhinum* were collected. Some of the commercial strains were obtained from wholesale seed companies located in California. Others were specially developed strains from four experiment stations in this country.

Practically all of the species, as distinguished from the commercials, were originally introduced by the United States Department of Agriculture, although some were purchased from seed houses, or obtained from private collectors. The sources of the various strains are indicated by the following abbreviations, used in tables 2 and 4:

Brus.: Botanic Gardens, Brussels, Belgium
Buch.: Botanic Gardens, Bucharest, Rumania
Germ.: Kaiser Wilhelm Institute, Berlin-Dahlem, Germany, via United States Department of Agriculture Division of Foreign Plant Introduction
Göt.: Göteborg, Sweden, via United States Department of Agriculture Division of Foreign Plant Introduction
Lom.: Collected by T. Little at Lompoc, California
Palm.: Palmero, Italy, via United States Department of Agriculture Division of Foreign Plant Introduction
Paris.: Museum of Natural History, Paris, France
Stock.: Botanic Garden, Stockholm, Sweden, via United States Department of Agriculture Division of Foreign Plant Introduction
T. & M.: Thompson and Morgan, Seedsmen, Ipswich, England
Turk.: Collected by the Westover-Wellman expedition in Turkey, received via United States Department of Agriculture Division of Foreign Plant Introduction
Vent.: Ventimiglia, Italy, via United States Department of Agriculture Division of Foreign Plant Introduction

Elaborate, detailed attempts to identify or verify the species in our trials has not been undertaken, although some self-evident examples of misnamed species have been corrected. For the most part, the species have been grown under the name under which they were received.

Of the commercial strains used, some were selected from the older standard rust-susceptible varieties (designated by "S" in the tables), in order to provide adequate checks for the resistant (designated by "R") strains and new species. A plus or minus sign following the "S" or "R" indicates "highly resistant" or "fairly resistant" as the case may be, as reported by the donor.

The seed for the strains tested in 1937 was sown in the greenhouse at

Davis in the winter of 1936–37 and carefully guarded from possible rust infection until the plants were large enough for transplanting; the young plants were then shipped to various localities in the state. Strains were tested at twelve localities, in 1937, but all strains were not grown at each locality. Eleven of these localities are listed in table 1. Unfortunately the trials at Eureka in the northern part of the state were a complete failure owing to lack of care, but the other trials were well cared for and gave very definite results.

The strains tested in 1938 were started under glass in Berkeley, where the same precautions were taken to prevent rust infection before transplanting, as were taken at Davis.

In 1937, twenty-five plants of each strain were used in the trials, except at Berkeley, Davis, and San Jose, where smaller numbers of certain strains were sometimes used because of insufficient plants, due to poor germination or other causes. The greatest number of accessions, however, were grown at the three localities just mentioned. These three places fairly well covered the range in climate from the relatively hot interior Sacramento Valley at Davis to the cool coastal situation at Berkeley, with an intermediate climate at San Jose.

Because the rust reaction of the various snapdragon strains had been similar in Berkeley and San Jose to what it was in the coastal and inland areas, respectively, in 1937, the trials were restricted to these two localities in 1938. Further reason for thus restricting the trials was that as much information regarding rust reaction could be obtained from smaller numbers of plants.

The plantings at Berkeley were thoroughly inoculated with a mixture of the rusts growing in the Salinas Valley and at Berkeley; in 1937 the inoculations were made twice, first after the plants were about half grown and again just before blooming; and in 1938 once only, just before blooming. No inoculations were made at any of the other localities; the plants were grown normally in the garden or field and were given no special treatment.

All trials were examined at least three times during the season. The ratings with respect to variability and rust reaction are generally those last observed. Whenever the variability was great—that is, 3—the *amount* of rust given represents the approximate average of all plants in that row at the time the observation was recorded.

RESULTS OF TRIALS

A comprehensive summary of the results of these trials is given in tables 1 and 2, for the 1937 trials, and in tables 3 and 4 for the 1938 trials.

With the exception of Berkeley mentioned above, these tables give the

TABLE 1

RUST REACTION OF DIFFERENT STRAINS OF ANTIRRHINUM MAJUS IN ELEVEN LOCALITIES, 1937

Source of seed and strain no.	1937 Culture no.	Berkeley		Davis		San Jose		Santa Maria		Guadalupe		San Diego		El Monte		Lompoc		Salinas		Sacramento		Chico	
		Amount*	Variability†	Amount*	Variability†	Amount*	Variability†	Amount*	Variability†	Amount*	Variability†	Amount*	Variability†	Amount*	Variability†	Amount*	Variability†	Amount*	Variability†	Amount*	Variability†	Amount*	Variability†
California Agr. Exp. Sta.:																							
1S	1	3	1	—‡	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
2R	2	1	1	0	1	4	2	0	1	10	1	5	1	5	1	3	2	10	2	0	1	0	1
4R	3	0	1	0	1	10	1	—	—	10	1	—	—	5	1	2	2	10	1	0	1	—	—
Univ. of Michigan:																							
1R	4	6	2	0	1	8	1	9	2	10	1	3	2	6	2	0	1	9	1	0	1	0	1
2R	5	—	—	0	1	2	1	—	—	10	1	—	—	—	—	—	—	9	1	—	—	—	—
3R§	6	3	2	0	1	—	—	—	—	—	—	—	—	—	—	6	1	—	—	—	—	—	—
4R	7	5	2	0	1	2	3	6	2	10	2	2	1	10	1	1	1	7	2	0	1	0	1
Michigan Agr. Exp. Sta.:																							
1R'35	8	3	2	0	1	0	1	—	—	—	—	—	—	2	1	—	—	—	—	2	1	—	—
1S'36																							
2R	9	1	1	0	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
3R	10	2	1	0	1	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
4R	11	1	1	0	1	1	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Massachusetts Agr. Exp. Sta.:																							
1R—	12	3	2	0	1	5	1	—	—	10	1	—	—	—	—	—	—	—	—	—	—	—	—
2R—	13	1	1	0	1	2	1	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
3R—	14	3	2	0	1	2	2	3	1	10	2	2	1	5	2	0	1	7	1	0	1	0	1
Ferry-Morse Seed Co.:																							
1S	15	9	2	0	1	9	3	—	—	—	—	—	—	10	1	1	2	—	—	—	—	—	—
2R	16	4	1	0	1	3	2	6	1	10	1	1	1	8	2	3	3	8	2	0	1	0	1
3R	17	4	1	0	1	0	1	2	2	10	1	2	1	3	2	1	1	—	—	0	1	0	1
4R	18	7	1	0	1	4	1	9	2	10	1	2	1	2	1	2	1	9	2	0	1	0	1

Waller-Franklin Seed Co.:																						
1R	19	6	2	0	1	4	1	8	1	10	1	8	2	2	1	3	2	9	2	0	1	0	1
2S	20	8	1	0	1	9	2	1	2	—	—	—	—	5	1	—	—	—	—	—	—	—	—
3R¶	21	2	2	0	1	1	1	2	2	10	2	2	1	4	2	1	1	4	1	0	1	0	1
4R	22	2	1	0	1	1	1	1	1	—	—	1	1	1	2	0	1	1	1	0	1	0	1
Macdonald Seed Co.:																							
1S	23	9	1	0	1	10	3	—	—	—	—	4	2	—	—	—	—	10	1	—	—	0	1
2R	24	5	2	0	1	2	2	2	2	10	1	10	1	7	3	2	3	6	2	0	1	0	1
3R	25	4	2	0	1	3	2	—	—	10	1	2	1	5	1	1	1	7	2	0	1	0	1
4R	26	4	1	0	1	2	1	1	2	10	1	2	1	4	2	1	1	8	2	0	1	0	1
Bodger Seeds Ltd.:																							
1R+	27	8	1	0	1	5	1	4	1	10	1	2	1	8	1	1	2	10	2	0	1	0	1
2R−	28	8	1	0	1	4	3	8	1	10	1	6	2	—	—	4	2	9	2	0	1	0	1
3R−	29	5	2	0	1	4	1	2	1	10	1	4	2	—	—	1	1	6	2	0	1	0	1
4S	30	9	1	0	1	10	2	9	1	—	—	6	2	—	—	—	—	—	—	3	1	0	1
Burpee Seed Co.:																							
1R	31	9	1	0	1	9	3	8	2	10	1	4	2	7	2	9	1	9	1	4	2	0	1
2S	32	9	1	0	1	10	1	—	—	—	—	1	1	—	—	—	—	8	1	2	2	0	1
3R	33	5	1	0	1	3	3	3	2	10	1	4	2	7	2	3	2	8	1	0	1	0	1
4S	34	9	1	0	1	9	2	9	3	10	1	—	—	10	1	—	—	—	—	—	—	—	—
5R	35	9	2	0	1	3	1	7	2	10	1	4	2	2	2	8	2	10	2	0	1	0	1
6R	36	8	2	4	2	9	2	7	1	10	1	7	2	4	2	—	—	8	2	1	2	0	1
7R§	37	6	2	0	1	6	1	2	1	10	1	2	1	4	2	1	2	8	2	2	2	0	1

* Amount of rust reaction ranges from 0, no rust, to 10, the plants either covered with rust or killed by it.
† Variability ranges from 1, uniform reaction of all plants within the strain, to 3, almost clean and heavily rusted plants in the same strain.
‡ Dashes indicate that the strain or species was not on trial in that locality.
§ Withstood the heat well at Davis and Sacramento.
¶ This line showed good resistance to "wilt" (pathogen not determined) at Davis and Sacramento.

TABLE 2

RUST REACTION OF DIFFERENT SPECIES OF ANTIRRHINUM IN ELEVEN LOCALITIES, 1937

Species, culture no., and source of seed*	Berkeley		Davis		San Jose		Santa Maria		Guadalupe		San Diego		El Monte		Lompoc		Salinas		Sacramento	
	Amount*	Variability†	Amount*	Variability†	Amount*	Variability†	Amount*	Variability†	Amount*	Variability†	Amount*	Variability†	Amount*	Variability†	Amount*	Variability†	Amount*	Variability†	Amount*	Variability†
Antirrhinum Asarina L.																				
No. 38, Buch.	F§	..	..	..	..	..	..	..	..	..	..	.	..	..	..	..	..	..	..	..
No. 39, Göt.	F	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
No. 40, Paris	F	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
No. 41, T. & M.	0	1	—¶	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
A. Coulterianum Benth.																				
No. 42, Brus.‖	—	—	0	1	D**	D	—	—	—	—	—	—	—	—	—	—	—	—	—	—
No. 43, T. & M.	F	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
A. chrysothales																				
No. 44, Paris	0	1	0	1	5	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
A. glandulosum Lindl.																				
No. 45, T. & M.	—	—	0	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
A. glutinosum Boiss.																				
No. 46, Buch.	9	1	0	1	10	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
No. 47, T. & M.	F	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
A. hispanicum Chav.																				
No. 48, Buch.	8	1	0	1	9	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—
No. 49, Paris††	4	1	—	—	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
A. latifolium D. C.																				
No. 50, Paris‡‡	—	—	—	—	10	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—
No. 51, Vent.	9	1	0	1	10	2	—	—	9	2	2	1	5	1	3	2	10	1	5	1
A. maurandioides Gray																				
No. 52, Brus.	—	—	0	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
No. 53, Buch.§§	1	1	0	1	0	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
No. 54, Paris	2	1	0	1	0	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—

A. molle L.																			
No. 55, Buch.	F	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
No. 56, T. & M.	2	2	0	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
A. meonanthum Hffgg.																			
No. 57, Paris	—	—	0	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
A. Orontium L.																			
No. 58, Brus.¶¶	—	—	0	1	D	D	0	1	1	1	D	D	D	D	0	1	0	1	D	D
No. 59, Göt.	—	—	0	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
No. 60. Palm.	—	—	0	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
No. 61, Paris	—	—	0	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
No. 62, Vent.	1	1	0	1	0	1	0	1	1	1	1	1	0	1	0	1	1	1	0	1
A. sempervirens Lapeyr.																				
No. 63, T. & M.‖‖	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
A. siculum Ucr.																				
No. 64, Buch.	1	1	—	—	4	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
No. 65, Palm.	F	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
No. 66, Paris	2	1	0	1	6	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
A. tortuosum Bosc.																				
No. 67, Palm.	2	1	0	1	8	4	3	1	6	3	1	1	3	2	1	1	5	1	0	1
No. 68, Turk.	9	2	0	1	10	1	10	3	10	2	4	1	8	3	7	3	10	1	5	1

* See text, p. 570 for explanation of abbreviations of sources.
† Amount of rust reaction ranges from 0, no rust, to 10, the plants either covered with rust or killed by it.
‡ Variability ranges from 1, uniform reaction of all plants within the strain, to 3, almost clean and heavily rusted plants in the same strain.
§ F indicates failure of the seed to germinate or failure of seedlings before transplanting.
¶ Dashes indicate that the strain or species was not on trial in that locality.
‖ Typical *A. Orontium;* not *A. Coulterianum.*
** D indicates plants died in the field before observations could be made.
†† Typical *A. majus;* not *A. hispanicum.*
‡‡ Probably *A. majus;* not *A. latifolium.*
§§ Not *A. maurandioides* Gray.
¶¶ Different form of *A. Orontium* from others in trial.
‖‖ Only one plant reached maturity; it showed medium infection at Berkeley.

reactions of the various strains under natural conditions at the localities where they were tested.

Although certain reservations and qualifications should be made in some cases, because all strains were not tried in all localities, and because of the necessary condensation of the information in the tables, yet, in

TABLE 3

Rust Reaction of Different Commercial Strains of Antirrhinum majus in Berkeley and San Jose, 1938

Sources of seed and strain no.	Culture no.		Berkeley		San Jose	
	1937	1938	Amount *	Variability†	Amount *	Variability†
California Agr. Exp. Sta.:						
2R	2	74	10	2	8	2
4R	3	79	10	1	5	2
1S	1	70	10	1	—‡	—
36-5-2-21 R	—	80	10	1	10	1
36-5-8-4 R	—	81	10	1	10	1
43-14-1-22 R	—	82	10	1	2	1
47-15-9-2 R	—	83	10	1	9	1
64-15-10-16 R	—	84	10	1	10	1
64-29-4-5 R	—	85	10	1	—	—
Univ. of Michigan:						
3R	6	72	10	1	8	1
4R	7	77	10	1	6	1
Michigan Agr. Exp. Sta.:						
2R	9	71	10	1	6	1
3R	10	78	4	1	3§	1
4R	11	73	3	1	3	1
Massachusetts Agr. Exp. Sta.:						
1R	12	76	10	1	4	1

* Amount of rust reaction ranges from 0, no rust, to 10, the plants either covered with rust or killed by it.

† Variability ranges from 1, uniform reaction of all plants within the strain, to 3, almost clean and heavily rusted plants in the same strain.

‡ Dashes indicate that the strain or species was not on trial in that locality.

§ Rust pustules mostly on seed pods.

summarizing the two years' trials as a whole and speaking in general terms, the following statements seem justified.

Susceptibility of Strains Tested.—No commercial strain tested where conditions for infection were severe, was found to be immune to rust. Some strains of certain species appeared to be immune during the limited trials to which they were subjected, but it is by no means certain that these same strains would not have become infected had it been possible to subject them to more extensive trials, or to the more severe conditions encountered at Guadalupe.

Several strains, however, both of commercial selections and of distinct

TABLE 4

RUST REACTION OF DIFFERENT SPECIES OF ANTIRRHINUM IN BERKELEY AND SAN JOSE, 1938

Species, culture no., and source of seed*	Berkeley		San Jose		Species, culture no., and source of seed*	Berkeley		San Jose	
	Amount†	Variability‡	Amount†	Variability‡		Amount†	Variability‡	Amount†	Variability‡
Antirrhinum Barrelieri Bor.					*A. latifolium* D. C.				
No. 87, Germ.§	9	1	—¶	—	No. 104, Germ.	10	1	—	—
No. 88, Germ.§	10	1	—	—	No. 105 Germ.	10	1	—	—
A. calycinum Lam.					*A. Linkianum*				
No. 89, Germ.	D‖	D	—	—	No. 106, Germ.	10	1	D	D
A. Charidemi Lge.					*A. litigiosum*				
No. 90, Germ.	2	1	—	—	No. 107, Germ.	6	1	—	—
A. chrysothales					*A. majus L.*				—
No. 91, Germ.	F**	..	..	..	No. 108, Germ.	3	1	—	—
A. glandulosum Lindl.					No. 109, Germ.	9	1	—	—
No. 86, Lom.††	0	1	0	1	No. 110, Germ.	10	1	—	—
A. glutinosum Boiss.					No. 111, Germ.	10	1	—	—
No. 92, Germ.	10	1	—	—	No. 112, Germ.	10	1	—	—
No. 93, Germ.‡‡	2	1	—	—	No. 113, Germ.	10	1	10	1
No. 94, Germ.	8	1	—	—	No. 114, Germ.	6	1	—	—
No. 95, Germ.‡‡	2	1	—	—	No. 115, Germ.	6	1	—	—
No. 96, Germ.	5	1	—	—	No. 116, Germ.	10	1	10	1
No. 97, Germ.	10	1	—	—	No. 117, Germ.	8	1	—	—
No. 98, Germ.	9	1	—	—	No. 118, Germ.	10	1	—	—
No. 99, Germ.	5	1	—	—	No. 119, Germ.	10	1	—	—
No. 100, Germ.	4	1	—	—	No. 120, Germ.	8	1	—	—
No. 101, Germ.	9	1	—	—	*A. meonanthum* Hffgg.				
A. hispanicum Chav.					No. 121, Germ.	10	1	—	—
No. 102, Germ.§§	10	1	—	—	*A. molle* L.				
No. 103, Germ.	F	..	..	..	No. 122, Germ.	F	..	..	..
A. Ibanjezii Pau.					No. 124, Germ.	F	..	..	..
No. 131, Germ.	2	1	—	—	*A. Orontium* L.				
No. 132, Germ.	8	1	10	1	No. 126, Germ.	0	1	—	—
No. 133, Germ.	2	1	—	—	*A. sempervirens* Lapeyr.				
No. 134, Turk.	F	..	..	..	No. 127, Germ.	F	..	..	..
No. 135, Turk.	10	1	—	—	*A. siculum* Ucr.				
No. 136, Turk.¶¶	10	1	10	1	No. 128, Germ.	2	1	3	1
No. 137, Turk.	8	1	10	1	No. 140, Stock.	4	1	1	1
No. 138, Turk.	8	1	10	1	*A. tortuosum* Bosc.				
No. 139, Turk.	5	1	3	1	No. 129, Germ.	F	..	..	..
					No. 75, no. 67 in 1937 trials	10	1	4	1
					A. valentinum F. Qu.				
					No. 130, Germ.	5	1	—	—

* See text, p. 570, for explanation of abbreviations of sources.
† Amount of rust reaction ranges from 0, no rust, to 10, the plants either covered with rust or killed by it.
‡ Variability ranges from 1, uniform reaction of all plants within the strain, to 3, almost clean and heavily rusted plants in the same strain.
§ These two strains are quite different things.
¶ Dashes indicate that the strain or species was not on trial in that locality.
‖ D indicates plants died in the field before observations could be made.
** F indicates failure of the seed to germinate or failure of seedlings before transplanting.
†† Collected in its native habitat.
‡‡ Not *A. glutinosum*.
§§ Doubtful identity.
¶¶ Looks like *A. majus, nanum grandiflorum* type.

species, were found to exhibit considerable resistance; so much so, in fact, that apparently little injury was sustained with respect to the seed set, although the plants themselves in some cases were noticeably infected. By comparing results for the different localities and, where possible, for the two years, it may be observed from the tables that the most outstanding lines in this respect are, among the commercials, Michigan Agricultural Experiment Station's nos. 3 and 4, and Waller-Franklin's nos. 3 and 4. Among the species, the most outstanding in resistance and seed set proved to be:

Antirrhinum Asarina L., culture no. 41
A. chrysothales, culture no. 44
A. glandulosum Lindl., culture no. 86
A. maurandioides Gray, culture nos. 53 and 54
A. Orontium L., culture nos. 58 and 62
A. Ibanjezii Pau., culture no. 133
A. siculum Ucr., culture no. 128

These species, however, are so far removed taxonomically from *Antirrhinum majus* that it is doubtful whether they will be of much value in a breeding program.

It is interesting to compare this list of resistant species with those enumerated by Mains.[9] He also found *Antirrhinum Ibanjezii, A. Asarina,* and *A. maurandioides* highly resistant; but *A. Orontium,* which he found "moderately resistant," was found to be uniformly highly resistant in these trials. His strain of *A. Barrelieri* was susceptible, as were both our strains, but he reports *A. glandulosum* as being susceptible, whereas we found the native *A. glandulosum* completely immune in 1938. Also the differences in rust reaction between the various strains of the different species, noted by Mains, showed up prominently in our trials. This was especially noticeable with *A. siculum* in 1937 and 1938 and with *A. Ibanjezii* and *A. glutinosum* in 1938. Also the differences in rust reaction between the various strains of the different species, noted by Mains, showed up prominently in our trials. This was especially noticeable with *A. siculum* in 1937 and 1938 and with *A. Ibanjezii* and *A. glutinosum* in 1938.

Effect of Climate on Rust Reaction.—Until fairly late in the season of 1937—after the middle of September—practically no rust was found on *any* of the strains in trials at Davis and Chico, and only a little on some strains at Sacramento. Apparently the low humidity and high temperature, such as were encountered there that season, were not conducive to rust development. After cool weather set in, however, there appeared an almost perfect differentiation in the three Sacramento Valley localities: strains which had been sent to us as "resistant" were *immune,* while

[9] Mains, E. B. Rust resistance in *Antirrhinum.* Phytopathology 25(11):977–91. 1935.

"susceptible" strains were all rusted. None of the wild species sent us were designated "susceptible" or "resistant," but the rust reaction discussed here was consistent with that in other localities for the wild species that were tried. Although infection was much less serious at Chico and at Davis than at Sacramento, comparable rust reactions of the various strains were plainly consistent. From this it seemed clear that there was only one rust strain present in these three localities during the 1937 trials.

The relation between temperature and humidity and extent of rust infection and damage was also noticeable in the 1938 trials at Berkeley and San Jose. In only two cases, those of *Antirrhinum Ibanjezii* and *A. siculum,* were there heavier infections at San Jose than there were at Berkeley, in all others, infections were the same or lighter at San Jose, where temperatures are higher and humidity lower.

Severity of Test at Guadalupe.—The heaviest infection in 1937 was at Guadalupe. Most of the strains were killed in this trial, and those that did survive until late in the season were badly injured. From the standpoint of testing rust resistance, this locality also proved to furnish the most severe test of any of the eleven localities where trials were grown. Mains (see footnote 9) also mentions that Guadalupe provided a very severe test for rust reaction in 1929.

But right here an interesting yet unsolved problem arises. In 1936, surveys made during field trips by the authors showed that rust infection at Guadalupe was very light while at Lompoc, some 30 miles south, the rust infection was so heavy that *Antirrhinum* seed crops were seriously damaged.

Yet, in 1937, the year these trials were grown in the two localities, conditions of rust infection were completely reversed: Lompoc showed an extremely light infection, while the crops at Guadalupe were damaged severely. Why the rust epidemic should vary so from year to year and be opposite in extent in two localities so close to each other and with such similar climates, is not known.

Evidently other forces than those exerted by hereditary factors play a rôle in determining the relative severity of rust infection.

Consistency of Rust Reaction.—The behavior relative to rust reaction of any given strain, as compared with others used in these trials, was fairly consistent in the different localities, with the exceptions noted above in the trials at the three Sacramento Valley localities. That is, a strain which was highly resistant at Berkeley or Guadalupe gave a very similar reaction in all other places, while the very susceptible strains showed approximately the same degree of susceptibility wherever they were grown except at Davis, Sacramento, and Chico, where only the

"susceptible" commercials showed any rust and they only late in the season. The fact that only the "susceptible" strains became infected late in the season at Davis, Sacramento, and Chico, but that this infection was heavy indicates that only the earlier known form or the more virulent form of rust was present in the Sacramento Valley, and that it did little damage until the weather became cool enough for it to become established.

Comparison of Rust Reaction with Seed Yields.—Fairly heavy seed yields were obtained in some strains in spite of rather severe rust infection, while very poor yields were obtained in other cases with a fairly high degree of resistance. Poor seed yield associated with a high degree of resistance was especially noticeable in several strains obtained from experiment stations. Apparently those strains had been selected for resistance to the rust with little regard to the factors concerned with seed yield.

Wind as a Factor in Spread of Rust.—Results of the trials indicate that wind aids rust dissemination to a very great extent. This is shown by the fact that in all trials the rust infection was greater on the leeward side, away from the wind, and noticeably less on the windward side, towards the wind; and this statement seems to hold, whether for single plants, single rows, or for a series of rows.

DISCUSSION

The occurrence of two or more forms of *Antirrhinum* rust is what one might readily suspect in the light of the difficulties encountered by the cereal breeders in producing rust-resistant small grains. But why the additional form of rust did not appear until approximately three years after several so-called "resistant" strains of snapdragon had been developed and introduced, and why it then became noticeable only in certain localities along the coast—that is, at Salinas, Guadalupe, and Lompoc—is a perplexing problem. This phenomenon might be accounted for on the basis of mutation or by hybridization among the rust fungi, and this explanation seems reasonable since the "newer" forms of rust were first observed at Salinas, then at Guadalupe, and later at Lompoc.

While discussing this problem early in 1937, Walter Lammerts of Ontario, California, presented what seems to be a plausible explanation. He was of the opinion that possibly the "newer" forms of rust had been present in these localities for some time, but had been unnoticed until the so-called "resistant" strains of snapdragon were introduced, because these rust forms were slower in action than those which so severely attacked the older-type, susceptible snapdragons. Yet if the various forms of rust had been prevalent in California for some time, there should not

have occurred the extremely noticeable differentiation among the *Antirrhinum* strains which was observed at Sacramento, that is, the "newer" forms of rust should have been present there also. On the other hand, the explanation suggested by Lammerts seems more likely because, in the most resistant strains of *Antirrhinum,* the rust apparently does not cause severe damage until late in the season, so late in fact, that it only slightly interferes with seed production.

Whatever the origin or mode of action of the different rust forms, the fact remains that from now on the development of rust-resistant commercial snapdragons does not present a simple problem in plant breeding.

SUMMARY

No commercial variety tested in two or more localities was found to be immune to rust (*Puccinia antirrhini* D. and H.), but several proved to be highly resistant.

The species *Antirrhinum Asarina, A. chrysothales, A. glandulosum, A. maurandioides, A. Orontium, A. Ibanjezii,* and *A. siculum* were found to be highly resistant. These species, however, are so far removed taxonomically from *A. majus* that it is doubtful whether they will be of much value in a breeding program.

The results indicate that the second form of rust has not yet appeared in the Sacramento Valley; possibly it is confined to the coast areas.

ACKNOWLEDGMENTS

We wish to express our thanks to the Division of Plant Pathology, and especially to Dr. M. W. Gardner, Dr. Ralph E. Smith, and to Dr. C. E. Yarwood of that Division for their generous coöperation on the problem. They have assisted in securing certain strains for trial, in providing land and labor, and in observing and rating the rust reaction of the trials.

We gratefully acknowledge the assistance of the following persons and institutions in providing seeds or land for use in these trials: Dr. E. B. Mains, University of Michigan; Professor Ray Nelson, Michigan State College; Professor Harold E. White, Massachusetts State College; Mr. B. Y. Morrison, United States Department of Agriculture Division of Plant Exploration and Introduction; Mr. J. C. Long, Plant Introduction Gardens, Chico, California; Mr. Dean F. Palmer, Agricultural Commissioner, Carlsbad, California; Mr. Thomas M. Little, Lompoc, California; Bodger Seeds, Ltd., El Monte, California; W. Atlee Burpee Company, Lompoc, California; Ferry-Morse Seed Company, Salinas, California; Lagomarsino & Sons, Sacramento, California; Waller-Franklin Seed Company, Guadalupe, California; and Wm. Macdonald Seed Company, Santa Maria, California.